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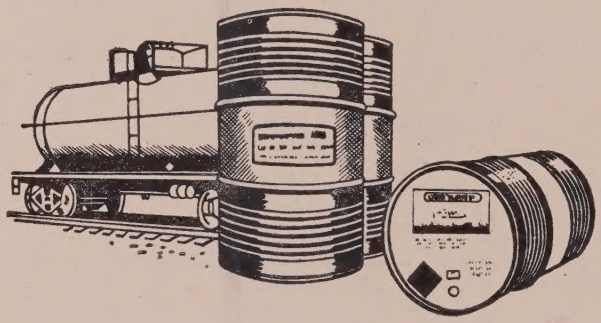

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HINDUSTAN ANTIBIOTICS Bulletin

Vol. 2 November 1959 No. 2

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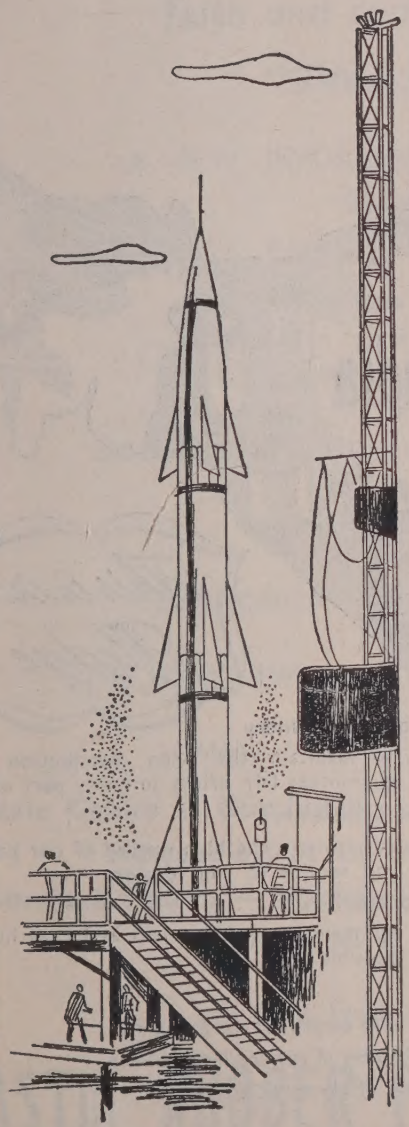

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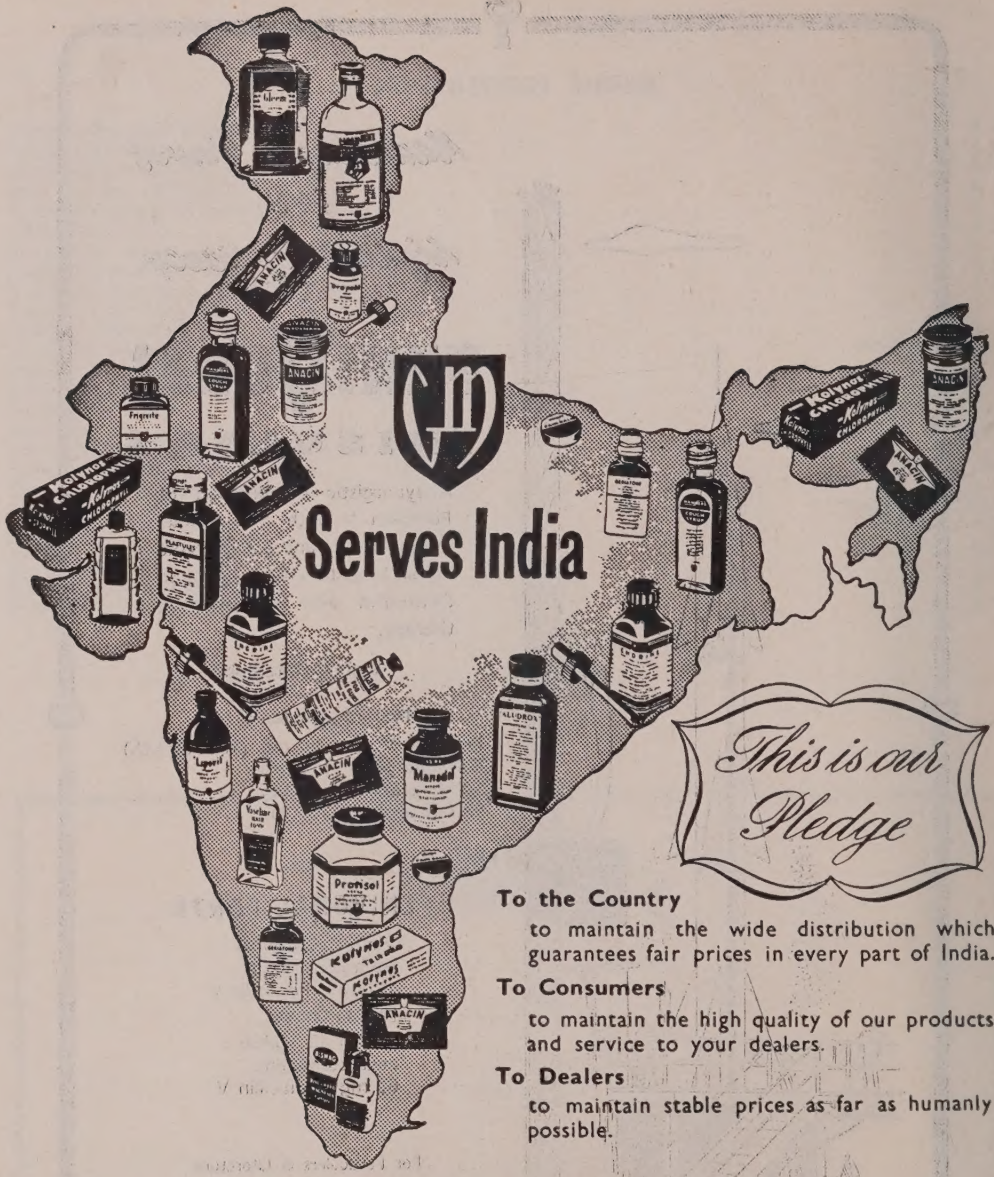

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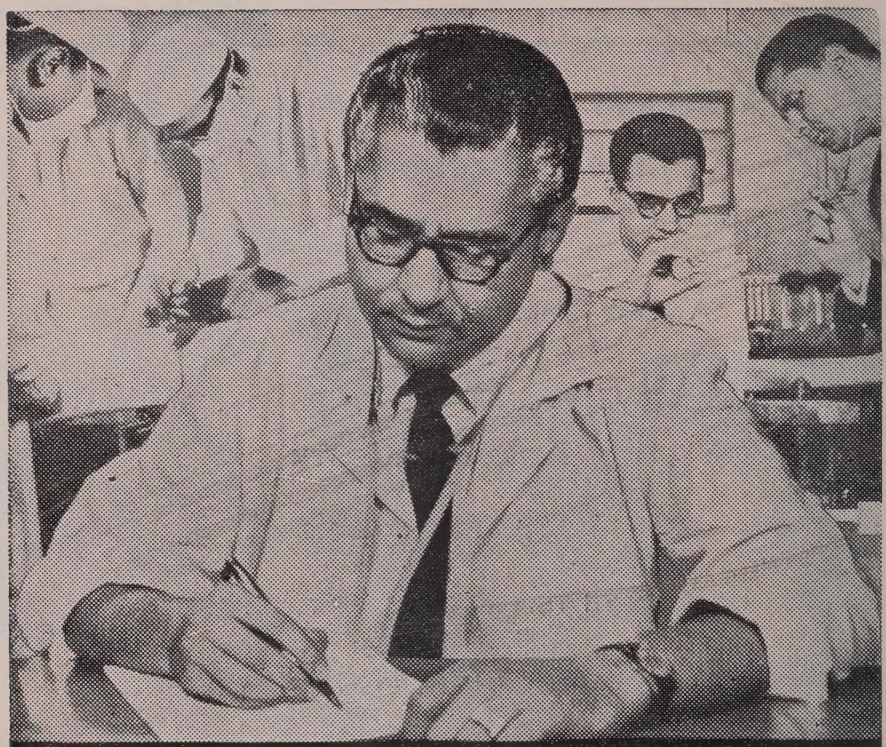

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Microbial Resistance to Antibiotics

In using antibiotics on a large scale for the control of human diseases, certain disadvantages have sharply come into focus. It has become apparent that some of the bacteria which were originally sensitive to the antibiotics, develop resistance as a result of continued contact with the drug. By exposing human pathogenic bacteria to low concentrations of antibiotics such as penicillin, streptomycin, chloramphenicol and tetracyclines, it has been possible to obtain resistant strains. When penicillin was first introduced into medical treatment, almost all the strains of *Staphylococcus* were sensitive to the antibiotic, whereas at the present time, in some of the hospitals where large does of the drug are being used, nearly 75 per cent of the strains isolated from human infections are reported to be resistant to penicillin. The ability of *Staphylococcus* to develop resistance to each of the new antibiotics discovered and used in therapy, has become a baffling problem. It has kept the microbiologists and chemists continuously busy looking for new antibiotics.

The drug resistance in the case of tubercle bacilli is even more alarming. Streptomycin and dihydrostreptomycin have long been the mainstay in effective control of tuberculosis. The rapid appearance of resistant strains has become a dangerous public health problem and this is being counteracted only by combined drug therapy.

The exact mechanism of the development of resistant strains varies in each case depending upon the mode of action of the antibiotic. In those cases where the organisms have inherited a resistant character prior to contact with the antibiotic, it is attributed to mutation. The mutants which arise, are subjected to the selective mechanism of the antibiotic. This results in the rapid multiplication of the resistant strains and disappearance of the susceptible ones.

The pattern of development of resistance may be step-wise as in the case of organisms developing resistance to penicillin, chloramphenicol and tetracyclines. In these cases, the resistance is of a low order to start with and builds up by continued exposure to the antibiotic. In contrast, streptomycin-induced resistance may follow all-or-none resistance type. It is believed that in the first type, the resistance determining factors are polygenic and in the second monogenic.

The theory of non-genetic type of resistance, usually termed "acquired resistance", is of extreme interest and much debated. Under the continuous stimuli of the drug in sublethal doses, the organisms are stated to adapt themselves and acquire resistance by different mechanisms which are often difficult to differentiate. The following postulates are put forth to explain the mechanism of acquired resistance in some of the cases studied:

a) Decreased permeability of the cell membrane of the pathogen to the drug.

b) Development of enzymes (like penicillinase) which destroy or inactivate the antibiotic.

c) Increased synthesis of the enzyme inhibited by the drug.

d) Development of alternate metabolic pathway and thus circumventing the inhibitory action.

e) Altered enzyme system which can still perform its metabolic function but is not affected by the drug.

These types of induced resistance are less permanent than natural resistance which is genotypic. Enzyme adaptation itself is reversible in the absence of the stimulating substrate.

It is now becoming evident that even when there is clear-cut evidence for genetic type of resistance, adaptive enzymes still play a part. If the newly acquired adaptive changes persist for a long time in the continued presence of the stimulating substance, they may get selected out by gene mutations. Even the addition of desoxyribonucleic acid from penicillin-resistant strains of pneumococci into the culture medium of the penicillin-sensitive cultures has been known to transfer genetic resistance. That the resistance-inducing genetic material could be transferred via bacteriophage from the resistant to susceptible strains, a phenomenon termed "transduction," has been demonstrated, thus providing yet another instance to show that our knowledge is far from complete to give definite answers to controversial problems.

Book Notices

Marti-Ibanez, Felix. Men, Molds, and History. New York, MD Publications Inc., 1958. 128 pp. $3.00.

Goldberg, H. S., *ed.* Antibiotics. Their Chemistry and Non-medical Uses. Princeton, N. J., D. Van Nostrand Co. Inc., 1959. 608 pp. $ 15.00.

Prescott, Samuel Cate, and Dunn, Cecil Gordon. Industrial Microbiology. 3rd. ed. New York, McGraw-Hill Book Co. Inc., 1959. 945 pp. $ 17.50.

Adams, Mark H. Bacteriophages. New York, Interscience Publishers Inc., 1959. 592 pp. $ 15.00.

Musilek, V., *ed.* Symposium on Antibiotics. Abstracts of Communications. Prague, 1959. 201 pp. + 33 pp. suppl. (Mimeographed).

Progress in Industrial Microbiology. Ed. by D. J. D. Hockenhull. London, Heywood & Co. Ltd., 1959. Vol. 1. 248 pp. 50 sh.

Annual Review of Biochemistry. Ed. by J. Murray Luck. Palo Alto, Calif., Annual Reviews Inc., 1959. Vol. 28. 698 pp. $ 7.50.

The Harvey Lectures. 1957-1958. New York, Academic Press Inc., 1959. Series 53. 254 pp. $ 7.50.

Advances in Biological and Medical Physics. Ed. by Cornelius A. Tobias and John H. Lawrence. New York, Academic Press Inc., 1958. Vol. 6. 640 pp. $ 16.50.

Veterans Administration. Armed Forces. 18th Conference on the Chemotherapy of Tuberculosis. February 2-5, 1959. Transactions. Washington D. C., 1959. 479 pp.

Penicillin in Obstetrics and Gynæcology

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With the advent of penicillin, systemic antibiotic therapy became available in 1942. It is difficult to overestimate the medical, public health and social benefits of antibiotics. The last decade witnessed a tremendous decline in maternal mortality and morbidity, the main contributive factors being the use of antibiotics in therapy and prophylaxis, and blood transfusion. In spite of the introduction of antibiotics of wider range of activity, penicillin has retained its predominent position in combating infections because of its efficacy, low toxicity and price.

Serum Concentrations and Dosage of Penicillin

Serum levels of penicillin following various regimens of dosage :

1. *Penicillin G*, single intramuscular dose :

Dose (units)	Level (units/ml.) at ....intervals after injection (hr.)			
	1-2	3	6	12
500,000 ..	20-25	4-6	1.0	0.03-0.25
300,000 ..	10-16	1.5-2.5	0-0.25	0
100,000 ..	3-6	0.01-0.3	0-0.01	—
20,000 ..	0.3-0.8	0-0.02	0-0.01	—

Usually 50,000 units 3-hourly or 0.1 million units 6-hourly are given in severe acute infections.

Continuous intramuscular or intravenous drip transfusion of a solution containing 300,000 units over a period of 24 hr. gives an average blood level of 0.1-0.54 units/ml. This method is not, however, generally used.

2. *Procaine penicillin :* Intramuscular injection of 300,000 units maintain a therapeutic concentration in blood for at least 24 hr. The maximum concentration is reached in 4 hr. and is lower than that produced by penicillin G. Procaine penicillin is usually used in mild or chronic infections.

3. *Procaine penicillin with 2% aluminium monostearate in oil* (*PAM*) gives adequate blood levels of penicillin upto 60 hr. This preparation is recommended in the treatment of syphilis.

4. *Dibenzylethylenediamine dipenicillin G* (*Benzathine penicillin*) *:* In severe infections, injection of 600,000 units every other day is recommended. For prophylactic purposes 600,000 units every two weeks or 1.2 million units every 4 weeks are advised. The oral dosage is 200,000 units 6-8 hourly.

5. *Phenoxymethylpenicillin* (*Penicillin V*)*:* Administered orally 125 mg. (200,000 units) 3 or 4 times a day maintain effective concentrations of the antibiotic in blood. Oral administration of Penicillin V is reserved for less severe conditions. For streptococcal infections without bacteraemia and in minor staphylococcal infections 200,000 units are recommended.

6. *N-benzyl - β -phenylethylamine salt of penicillin G.*: Injection of 300,000 units maintains adequate level of penicillin in blood for 3 days. 600,000 units can main-

tain it for more than 4 days. This preparation can be used orally.

A majority of the infective conditions in obstetrics and gynaecology are caused by penicillin sensitive organisms. The principal infective conditions resistant to the antibiotic are those incited by *M. tuberculosis, E. coli, Trichomonas vaginalis, Candida albicans* and other pseudo-yeasts, Ducrey's bacillus and Donovan bodies, virus of lymphogranuloma inguinale, *B. crassus* and *Entamoeba histolytica.* In severe infections, along with penicillin other antibiotics especially streptomycin, and sulphonamides would be beneficial.

Penicillin has no effect on menstruation, pregnancy and lactation.

OBSTETRICS

During pregnancy, apart from the treatment of intercurrent infections, penicillin is of value in the treatment of syphilis. A history of repeated abortions in the middle trimester, premature labours or still births may be indicative of syphilitic conditions in the mother. Penicillin is sometimes administered in such cases even when serological tests are negative and good results have been reported. However, such claims should be examined in the light of the fact that beneficial results might have been obtained without any treatment of the cases. Pregnant women suffering from syphilis should be treated promptly and at all stages of pregnancy. A repetition of the penicillin course in each succeeding pregnancy between the third and fifth months is advised. Where ample facilities are available for regular month to month check up of the patient, penicillin alone would be the drug of choice. Unfortunately in our country a follow up of the patient is rarely possible so that in potential mothers and young children at least one course of arsenic and one of bismuth and preferably two courses of each are advisable. If arsenic cannot be risked at least bismuth should be given with penicillin. There is some evidence that the combination has synergistic action.

Penicillin treatment has also been advocated in cases of toxaemia of pregnancy. It is supposed to neutralise a hypothetical toxin and induce diuresis.

Intranatal use of penicillin is mainly for prophylactic purposes. Prolonged labour, anaemia, haemorrhages and toxaemia of pregnancy diminish woman's resistance to infection. Prolonged labour with early rupture of membranes poses the serious problem of infection of liquor amnii. Inhalation of the liquor leads to pneumonia in foetus. Prolongation of labour even with intact membranes can lead to uterine sepsis. Any operative interference necessary during labour increases risk of infection. Vagina harbours pathogenic organisms and any interference carries them upwards to the uterus. Hence, in cases of premature ruptures of membranes, prolonged labour and when an operative delivery is anticipated prophylactic use of penicillin is indicated. Penicillin is sometimes introduced into the peritoneal cavity during Caesarian section as a prophylactic measure against suspected infection. Penicillin administered to the mother passes to the foetus and the liquor amnii thus affording a certain degree of protection to the foetus.

Puerperal sepsis has been the bugbear of obstetricians for centuries. Prior to the advent of penicillin 33 per cent of maternal mortality was due to sepsis. In the larger cities provision of adequate antenatal care, tendency to have delivery in hospital and use of antibiotics in therapy and prophylaxis has greatly diminished the incidence of this complication. There are, however, cases in which the danger of infection is a very real one. The main organisms causing postabortal and puerperal infection are : Anaerobic streptococcus, aerobic non-haemolytic streptococcus, *B. coli, Staphylococcus albus,* anaerobic staphylococcus, *Streptococcus viridans, B. welchii, a*-haemolytic

streptococcus and *Staphylococcus aureus*. Apart from placental infection, lacerations of vulva, vagina and cervix may get infected by pyogenic organisms. In puerperal sepsis massive doses of penicillin along with sulphonamides and other antibiotics are recommended. Ideally detection of the causative organisms and determination of their sensitivity to antibiotics should be done prior to treatment. A significant point of value in penicillin therapy in obstetrics is that we are able to take a certain degree of risk of maternal sepsis when such acceptance would be advantageous to the baby. In these circumstances prophylatic use of penicillin would normally prevent development of septic complications in the mother.

Extragenital infections in pueperium such as mastitis, thromboplebitis and pyelonephritis are also amenable to penicillin treatment.

Infection of uterus following septic abortion is more common and serious than infection after full-term delivery. It is the chief cause of abortion mortality, being responsible for about three-quarters of all such deaths. Infection is usually streptococcal and at times it can be so virulent that it fails to localize resulting in fatal general peritonitis. The advent of penicillin and other antibiotics has greatly brought down the mortality rate from this complication.

Many infective conditions in the new born are successfully controlled with penicillin. Immediately after birth, drops of penicillin placed in the baby's eyes prevent ophthalmia neonatorum. Other common conditions in the new born amenable to penicillin treatment are pneumonia, umbilical sepsis, pemphigus, septicaemia, pyaemia, tetanus and congenital syphilis. In babies penicillin may be given orally for mild infections as there is no hydrochloric acid in their stomach so that penicillin is not destroyed before it is absorbed. Dosage is as follows :

	Total daily dose, units/lb. body weight/day	
	Mild infection	Severe infection
Intramuscular Injection		
Sodium or potassium Penicillin G	5,000-10,000	upto 2,000,000
Procaine penicillin ..	do.	upto 1,000,000
Oral		
Penicillin G ..	20,000	Not indicated
Procaine penicillin ..	do.	do.
Benzathine penicillin	do.	do.
Penicillin V ..	100,000-300,000 5 times a day	

Penicillin given to the mother passes into breast milk and hence to the baby.

GYNAECOLOGY

Main infective conditions met with in gynaecological practice are: (1) Chronic infective conditions left over from puerperal and post-abortal sepsis; (2) venereal diseases; (3) secondary infection of ulcerations due to (a) injuries, (b) foreign bodies, (c) carcinoma; (4) tuberculosis; (5) infections due to *Trichomonas vaginalis*, *Candida albicans* and other *Moniliae*, and *Entamoeba histolytica*; and (6) postoperative infections.

Venereal Diseases

Venereal diseases responding to penicillin are gonorrhoea and syphilis. In women manifestations of these diseases are much milder than in men and very often they are unaware of it until some complication of chronic stage sets in.

Gonorrhoea can be regarded as the best example of pelvic inflammation in women as it produces lesions of vulva, vagina, uterus, fallopian tubes, ovaries and the pelvic peritoneum.

Pencillin is the most widely recommended antibiotic for gonorrhoea. A single injection of 300,000 units of procaine penicillin is sufficient for complete cure of acute gonorrhoea involving the vulva. A disadvantage with penicillin treatment is the

masking of syphilitic infection if the latter has also been contracted at the same time. Therefore, a serological test for syphilis must be done after three months of treatment with penicillin of gonorrhoeal cases. Penicillin V, 125 mg. (200,000 units) every 4 hr., five to six doses, has proved effective.

Adult vaginal mucosa is resistant to gonococcal infection except in cases of infection with very virulent organisms, defloration and in children. In the latter, along with penicillin estrogens are administered to build up the vaginal mucosa.

Spread of infection upwards from cervix leads to inflammation of endometrium, fallopian tube and pelvic peritoneum and the patient is acutely ill. Massive doses of penicillin are recommended for such cases.

Chronic gonococcal infections are very resistant to treatment, and penicillin therapy should be supplemented by short wave diathermy and surgical procedures.

Syphilis becomes a generalised infection after the genital infection in the primary stage. The whole picture of syphilis treatment has changed with the advent of penicillin. The antibiotic can be administered in several ways. PAM is widely used and daily injections of 600,000 units of it for ten days are effective. Some prefer to give the injections at two or three intervals. Both the schedules are, however, equally efficacious. If penicillin G is used it is customary to give 60,000 units 3-hourly for a total of 90 injections. Benzathine penicillin : In primary and secondary syphilis, 2,400,000 units intramuscularly at first treatment, followed by four injections of 600,000 units each at 4-day intervals ; pregnancy, first or second trimester, 600,000 units twice weekly for 4 weeks or 1,200,000 units once a week for 4 weeks; pregnancy, third trimester, 600,000 units daily for 8 days ; when labour is imminent, 2,400,000 units at one time repeated in 1 week if patient is not delivered.

In infantile congenital syphilis, a total dose of 200,000 units of penicillin per pound of body weight are advised, the injections given three hourly for ten days. Repository forms of penicillin are also recommended. For cases exposed to syphilis, one injection of PAM, 600,000 units given within six hours of exposure affords protection against both syphilis and gonorrhoea. If 12 hours have passed two injections are necessary. Prophylactic treatment of syphilis after exposure to infection is not, however, advisable. The infection may remain masked without getting cured for many months by this short treatment so that patient has to be kept under observation for 2 years even though he may not have contracted the disease.

Chronic infections left over from postabortal and puerperal sepsis require supplementary treatment along with antibiotics.

After all gynaecological operations penicillin is given as a routine. For secondary infections of ulcerative conditions such as traumatic, tuberculosis, granulomatous or carcinomatous ulcerations, penicillin is used along with specific treatment.

The use of penicillin in obstetrics and gynaecology has thus greatly reduced maternal mortality and morbidity. Sequelae of puerperal and postabortal infections and gonnorhoea leading to chronic ill-health, backache, leucorrhoea and sterility can be controlled by timely use of the antibiotic. Although the subject of infection has for the time being lost its grimness, the present lull due to antibiotics may not continue indefinitely if the race between the development of resistance and the production of newer antibiotics ever goes in favour of the former. The possession of powerful weapons against infection is no excuse for disregarding preventive aspects of infection in obstetric and gynaecological practice.

Mycothricin Complex, A Streptothricin Group of Antibiotics*

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Among the various types of antibiotics produced by actinomycetes, the streptothricin group occupies a prominent place, both because of its production by a number of actinomycetes and because of the great variety of representative forms that have so far been isolated. Waksman and Woodruff reported in 1942 the isolation and characterization of the first representative of this group of basic antibiotics, streptothricin. Since then, several antibiotics of the streptothricin group have been isolated from actinomycetes. The streptothricin group of antibiotics are characterized by the following properties : (i) They are basic, water-soluble, and thermostable substances giving a positive ninhydrin reaction ; (ii) they are active against gram-positive, gram-negative and acid fast bacteria, in general, but less effective on the *Bacillus cereus-mycoides* group ; and (iii) they cause delayed toxicity when injected into mice. Further differentiation of the members of the group could be made on the basis of (a) antimicrobial spectra, especially antifungal and antiviral properties, and also their activity ratios against certain test organisms, (b) chemical properties, *e.g.*, hydrolytic products, composition and colour reactions, (c) physical properties, *e.g.*, optical rotation, melting point of derivatives, diffusibility in agar media and R_f mobility, and (d) toxicity levels in mice, rabbit's eye test, etc. Streptothricin, streptin, actinorubin, lavendulin, streptolin, pleocidin, roseothricin, geomycin, belong to this group.

Mycothricin complexes A and B were isolated by Rangaswami, Schaffner and Waksman in 1956 from two strains of *Streptomyces lavendulae*, from a soil sample collected from a wood. The two mycothricin complexes were found to be closely related to streptothricin in their chemical and physical properties and in their activity against micro-organisms, in general. But they were distinct from the other members of the group in their antifungal spectra, R_f values on paper chromatograms and in their hydrolytic products. The two complexes are closely related to each other in their physical, chemical and microbiological properties, but each of them comprise of complexes rather than single chemical entities and they differ in certain antimicrobial properties also.

Isolation and Characterization

A medium containing 2 per cent soya-peptone, 1 per cent cerelose and 0.25 per cent sodium chloride gave the best production of antibiotic when the cultures were grown in shake flasks at 28° on a rotary shaker. A maximum of 3,000 dilution units/ml. of the medium was obtained within three days in soya-peptone-cerelose medium as against 2,000 or less units/ml. in yeast extract-glucose nutrient, or soya bean meal-peptone medium. The influence of aeration on antibiotic production was tested by growing the cul-

* A major portion of the work reported here was carried out in the Institute of Microbiology, Rutgers State University, New Brunswick, N. J., under the guidance of Dr. S. A. Waksman.

tures in 250 ml. Erlenmeyer flasks containing various volumes of the medium under submerged aerated conditions. In both cases 75 ml. volume gave the best results, higher or lower volumes interfering with the production. The effect of temperature on antibiotic production was tested under the above submerged aerated conditions and it was found that both the cultures were capable of growing and producing the antibiotic under a wide range of incubation temperature, 20° to 37° ; the antibiotic production at 32° was more rapid than at 28° and at 37° only traces of the antibiotics were produced. In order to test the possibilities of producing the antibiotic in pilot plant scale one of the cultures was tested by comparing the antibiotic production in different containers. The results are given in the table below.

No.	Capacity (container)	Quantity of medium	Maximum unitage	Hours of fermentation
1	250 ml. (flasks)	.. 75 ml.	750	48-72
2	2 1. (flasks)	..600 ml.	400	72
3	5 1. (pyrex fermentors)	2.5 1.	625	33-40
4	40 1.. (stainless steel formentors)	25.1	400	36
5	300 gal. (stainless steel formentors)	90 1.	100	36

These studies clearly indicate the effect of aeration and the type of aeration on antibiotic production.

The mycothricin complexes were extracted from the fermented broths by the standard method of purifying streptothricin and related antibiotics, *viz.*, adsorption on to Darco-G60, elution with acid alcohol and concentration of the eluate. The concentrated crude preparation was further purified by converting it into crystalline helianthates and then to hydrochlorides when a colourless water soluble preparation was obtained.

The cultures were also grown in a synthetic medium containing 10 g. of glucose, 2.0 g. of potassium phosphate, 0.005 g. of hydrated zinc sulphate, 0.01 g. of hydrated ferrous sulphate, 0.005 g. of hydrated manganese sulphate, 0.5 g. of hydrated magnesium sulphate, 0.25 g. of sodium chloride, 10.0 g. of glutamic acid in a litre of distilled water. The antibiotic produced was extracted and purified as detailed above. This preparation was compared with that obtained from the complex organic medium on paper chromatograms developed with propanol-pyridin-acetic acid-water (15: 10: 3: 12), when certain differences in the components of the two complexes obtained from the two sources were observed, indicating the complex nature of the substances and the influence of the medium on the components of the complexes.

The two complexes were assayed in agar and liquid broth media under different pH, incubation temperature and glucose and sodium chloride concentration of the medium. Higher concentrations of yeast extract or glucose in the medium adversely affected the inhibition of test organism, whereas addition upto one per cent of NaCl appeared to enhance the antibiotic activity. The two complexes where more active in alkaline than in acid reactions of the substratum. One unit of the antibiotic was defined as the minimum amount required per ml. of nutrient agar for complete inhibition of growth of an 18-hour culture of *Escherichia coli* at 37°.

The two mycothricin complexes were compared with streptothricin, streptothricin VI, geomycin, viomycin, pleocidin, vinactin, streptomycin, neomycin and catenulin for relative activity against *Bacillus subtilis*, *B. mycoides*, *E. coli*, *Pseudomonas fluorescens*, *Candida tropicalis*, and *Saccharomyces cerevisiae*. The two complexes were more active against fungi than the other antibiotics tested, except pleocidin, which was much less active on *E. coli* and *B*

mycoides. There were distinct differences between the two complexes in the ratios of activity on the cultures tested. These results were further compared by paper chromatographic assay against *B. subtilis*, when the R_f values for the two complexes were found to be much lower than the related antibiotics.

The two mycothricin complexes are readily soluble in water at acid, neutral and alkaline reactions, but only slightly soluble in methanol and ethanol. They are insoluble in *n*-butanol, benzene, ether, petroleum ether, ethyl acetate, chloroform and acetone. Both complexes were more stable to heat in acid and neutral than in alkaline solutions. There was little loss of activity when heated for 10 minutes at 95° in acid and neutral reactions, but heated at that temperature in alkaline solutions there was 33 and 35 per cent inactivation of the complexes A and B, respectively. The complexes consist of strong organic bases, forming stable salts with various organic acids to give such derivatives as picrates, helianthates, flavianates and reineckates. The ninhydrin, Pauly, and biuret reactions were positive whereas the Fehling's Tollens', Molisch's, maltol, Sakaguchi, Millon's and Hopkins-Cole tests were negative or inconclusive.

Purified samples of the two complexes were hydrolysed with 6 *N* hydrochloric acid at 100° for 48 hours in sealed tubes and the hydrolysates from streptothricin, geomycin, pleocidin and viomycin were compared on paper chromatograms. Seven ninhydrin positive spots each were obtained with the two mycothricin complexes as against three or four spots in the other antibiotics tested.

Toxicity studies by subcutaneous injections of the two substances in mice gave LD_{50} values of 65 to 130 mg/kg. and 32.5 mg/kg. body weight for the complexes A and B, respectively. Certain delayed toxicity, characteristic of the streptothricin group, was also observed in both cases. Yellowing of the eye is another characteristic of some members of the streptothricin group, but in rabbit's eye test the two mycothricin preparations were found non-toxic.

Action on Plant Pathogens and Nematode

Mycothricin complex A inhibited most plant pathogenic bacteria and fungi tested at concentrations ranging from 1 to 10 mcg/ml. in agar dilution plates. By the spore germination method fungi were inhibited at still lower concentrations. Tested against a nematode, *Rhabditis briggsae*, in nutrient agar media with dead cells of *E. coli* and different concentrations of mycothricin complexes A and B, there was inhibition of growth and motility, resulting in the death of nematodes within five days, at the concentration of 10 and 100 mcg/ml. tested.

Use In Plants

The toxic effect of the antibiotics was tested on seeds and on plants. Soaking seeds of cucumber, tomato and wheat in aqueous solutions of the antibiotic for one hour at 5,000 p.p.m. did not affect the germinability of the seeds and also there were no apparent symptoms of chlorosis or stunting in the emerging seedlings. Seedlings of tomato, wheat and cucumber raised in pots were sprayed with various concentrations of mycothricin complex A at weekly intervals starting from the third week after sowing. No phytotoxic symptoms were observed even with 2,500 p.p.m. of the antibiotic. Tomato and cucumber plants sprayed with the antibiotic were assayed for the presence of the antibotic in the roots, stem and leaf. Sprayed on the foliage the antibiotic was absorbed within 4 hours, systemically translocated in the plant and could be detected in the stem and root after 18 hours.

The antibiotic was also tested for its effect in controlling seed-borne fungal

infection. Wheat seed, infected both internally and externally with *Helminthosporium* sp. causing pre- and post-emergence seedlings blight, were treated by soaking in 1,000, 2,500 and 5,000 p.p.m. of mycothricin complex A for 18 hours, washed with sterile water and plated on oatmeal agar media. At 2,500 and 5,000 p.p.m. there was complete inhibition of the fungal growth from the seeds, indicating the inhibitory effect of the antibiotic on internally seed-borne organism. Also, there was better germination of the seeds as compared to the untreated control. In a similar experiment on the internal seed-borne infections of *Xanthomonas malvacearum*, causing blackarm disease, the incitant was completely inhibited at 500 and 1,000 p.p.m. of the antibiotic. These results clearly indicate the possiblities of using the antibiotic for plant disease control.

Discussion

The streptothricin group of antibiotics are of importance because of some of their biological, physical and chemical properties such as water-solubility, thermostability and stability to light as well as easy production in large quantities by fermentation. Their wide antimicrobial spectra — some of the members possessing considerable antifungal effect — are of great significance. Unfortunately all these chemicals are relatively toxic for clinical use in man and animal. But in recent years increased attention is being paid to the use of antibiotics in agriculture. Of the various antibiotics studied for use in plant disease control streptomycin has proved highly effective against bacterial diseases of plants. This is mainly because of its water-solubilty, thermostability and systemic activity in plants. The members of the streptothricin group are closely related to streptomycin in their solubiltiy, thermostability etc., and added to this they have a wider antimicrobial spectra. The studies made so far with mycothricin clearly indicate its effectiveness on plant pathogenic bacteria and fungi, its systemic translocation in plants and its *in vivo* effect on the internally-borne fungal and bacterial infections of seeds. These studies are to be intensified and extended to the other members of streptothricin group so that some of them may prove to be of use in plant disease control.

REFERENCES

1. Rangaswami, G., Schaffner, C. P., and Waksman, S. A. Isolation and characterization of two mycothricin complexes. *Antibiot. and Chemother.* **6**, 675 (1956)

2. Rangaswami, G. In vitro effect of mycothricin on plant pathogenic bacteria and fungi. *Mycologia* **48**, 800 (1956)

3. Rangaswami, G. A preliminary report on the use of mycothricin in plants. *Plant. Dis. Reptr.* **40**, 483 (1956)

4. Rangaswami, G. Effect of some antibiotics on *Xanthomonas malvacearum* and on the microflora of cotton seed. *Indian Phytopath.* **10**, 8 (1957)

5. Rangaswami, G. Studies on the morphological, cultural and physiological properties of five antibiotic producing isolates of the *Streptomyces lavendulae* group. *Indian Phytopath.* **11**, 165 (1958)

6. Rangaswami, G. Nutrition of *Streptomyces lavendulae* in relation to antiboitic production. (Abs.) Indian Institute of Science, Golden Jubilee Symposia, August 1958 pp. 19-20.

7. Schaffner, C. P., Rangaswami, G., and Waksman, S. A. Mycothricin, a new polypeptide antibiotic of the streptothricin group. *Proc. Third Intern. Cong. Biochem.* Brussels, Belgium, 1955

8. Tiner, J. D., and Rangaswami, G. Effect of mycothricin complex on the nematode *Rhabditis briggsae*. *Proc. Helminth. Soc., Washington*, **24**, 1 (1957)

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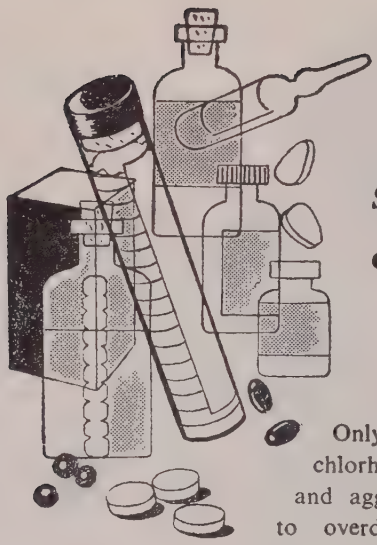

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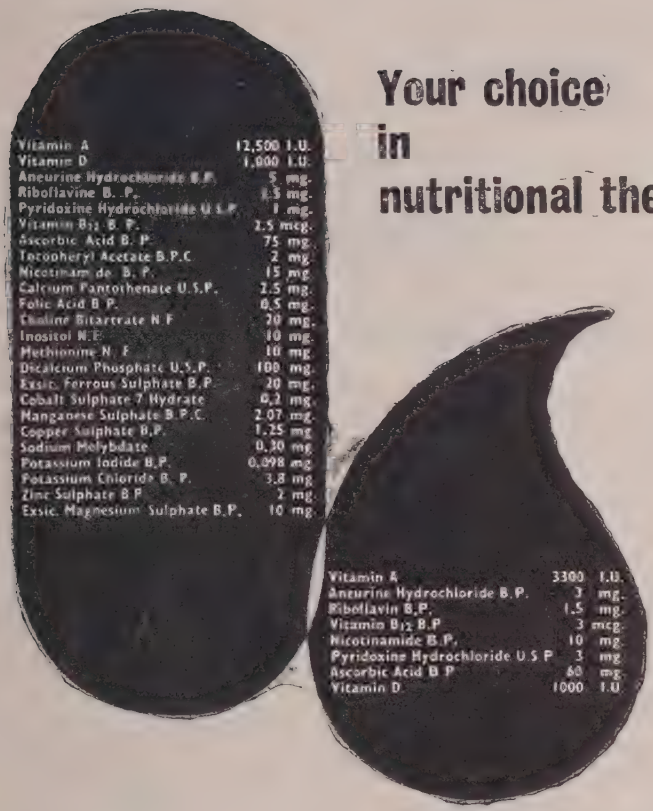

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Vitamin B_{12}: Some Aspects of its Chemistry and Assay in Natural Materials

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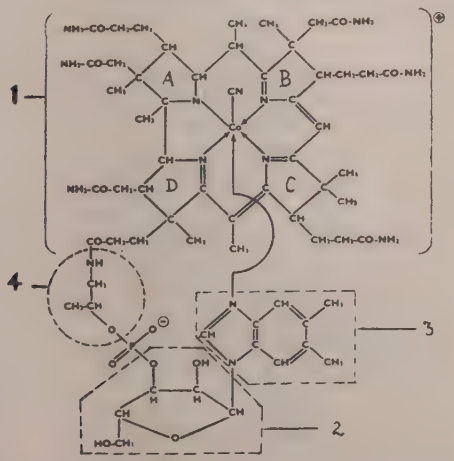
In 1926 a new chapter in the history of vitamins was opened when Minot and Murphy showed that whole animal liver is useful for treatment of pernicious anaemia. Following this discovery various groups of investigators began intensive work to isolate the "antipernicious anaemia factor" from animal liver. Finally in 1948, Rickes and others of Merck and Co., U.S.A., and Smith and co-workers of Glaxo Laboratories in England, almost simultaneously but independently, isolated this factor from liver in pure crystalline form and named it vitamin B_{12}.

STRUCTURAL FORMULA OF VITAMIN B_{12}
$C_{63}H_{90}N_{14}O_{14}PCo$



1. Porphin-like structure
   A, B, C, D — Pyrrole rings
2. L-ribose moiety
3. 5:6-Dimethylbenzimidazole group
4. *iso*Propanolamine

Chemical Structure

The complete chemical constitution of vitamin B_{12} was elucidated by Hodgkin and others of Oxford University, in 1956. The vitamin molecule contains a centrally located heavy cobalt atom present in the form of a co-ordination complex with a replaceable cyano group and is surrounded by a pattern closely resembling that of natural porphins. A side chain comprising of a dimethylbenzimidazole moiety; ribose and phosphate form a link between the central cobalt atom and the enclosing polypyrrole configuration.

The complexity of the molecular structure of vitamin B_{12} provides wide scope for the occurrence of a variety of closely resembling compounds. Two series of such compounds are known, series of cobalamins and series of vitamin B_{12}-like factors. The cobalamins are formed by a substitution of cyano group by various other groups and are, therefore, referred to by specific names depending upon the replacing group, *e.g.* hydroxocobalamin (or $B_{12}a$, $B_{12}b$, $B_{12}d$), nitrocobalamin ($B_{12}c$), thiocyanatocobalamin, chlorocobalamin, sulphatocobalamin, etc., true vitamin B_{12} being referred to as cyanocobalamin. The variants can be readily converted to cyanocobalamin by treatment with cyanide and are distinguished by their chromatographic behaviour and partition coefficients in different solvents.

Vitamin B_{12}-like factors, differ from each other and from vitamin B_{12} in the content of their nucleotide moiety.

Obviously therefore, and unlike cobalamins, these factors cannot be converted to vitamin B_{12} by treatment with cyanide. Purines and pyrimidines, and their derivatives, can replace the 5:6-dimethylbenzimidazole part of the vitamin B_{12} molecule resulting in the formation of factors like Factor A, Factor B, Factor C and pseudovitamin B_{12}. The number of vitamin B_{12}-like factors reported is very large, the important ones being those mentioned above. Of these, factor B does not contain any nucleotide and, therefore, can be regarded as the fundamental unit of vitamin B_{12} series and a link between cobalamins and vitamin B_{12}-like factors.

Assay

The complicated chemistry of vitamin B_{12} makes it difficult to obtain reliable data on the distribution and content of true vitamin B_{12} in natural materials. Vitamin B_{12} activity in natural materials may result from the presence to different extent, of cobalamins, vitamin B_{12}-like factors and other interfering materials. Higher animals are narrowly selective in their vitamin B_{12} requirement and the contaminating substances present in natural materials may have little or no clinical activity against pernicious anaemia or biological activity in animals. Moreover, there are few rich sources of the vitamin in nature and very commonly the vitamin occurs in such concentrations that error in estimation may invalidate the results to a large extent.

As additional information became available on the chemistry and physiology of the vitamin, a number of physical, chemical, microbiological and biological methods for assay were developed. Physical and chemical methods are based on (i) ultraviolet and visible absorption spectra, (ii) countercurrent distribution in different solvent-systems, (iii) quantitative liberation of the cyanide radical or the 5:5-dimethylbenzimidazole moiety by hydrolysis and its chemical estimation, and (iv) isotope dilution using C_O^{60}. All these physico-chemical methods, however, are limited in their application to relatively pure solutions of rather high potency such as pharmaceutical preparations.

Microbiological assay has been the most widely applied technique for vitamin B_{12} assay since the first report in 1947 by Shorb that *Lactobacillus lactis* Dorner is suitable for quantitative estimation of this vitamin. This organism was later abandoned in favour of certain others which are comparatively more specific and required fewer precautions. The most thoroughly tested method for vitamin B_{12} assay is the one adopted by U. S. Pharmacopoeia using the organism *Lactobacillus leïchmannii*. A shortcoming of this test organism is that its vitamin B_{12} requirement is partly spared by deoxyribosides of certain purines and pyrimidines (adenine, hypoxanthine, cytosine and thymine) as well as by intact deoxyribonucleic acid. It also responds to most of the B_{12}-like factors expect Factor B. Differential assay procedures have therefore been recommended in certain cases to eliminate interfering materials.

A vitamin B_{12} requiring mutant of *Escherichia coli*, isolated by Davis and Mingioli in 1950 by ultraviolet treatment, has also been widely adopted for assay of vitamin B_{12} by cup-plate or serial dilution method. The organism is slightly less sensitive than *L. leichmannii* and is subject to interference by methionine and B_{12}-like factors but not by deoxyribosides and deoxyribonucleic acid. The assay technique and media are quite simple and comparatively less time-consuming.

A highly sensitive method based on the B_{12} requirement of a photosynthetic algae *Euglena gracilis* var. *bacillaris*, first reported by Hutner and others in 1949, is now being successfully employed for assaying especially materials of low potency. The organism does not respond to deoxyribosides but

does so to B_{12}-like factors much in the same way as other assay organisms. Two more unfamiliar organisms recommended are the Chrysomonads *Ochromonas malhamensis* and *Poteriochromonas stipitata*. Both are brown-pigmented, photosynthetic, phagotrophic, flagellated protozoa and are notably indifferent to non-specific interfering materials and B_{12}-like factors. However, B_{12} requirements of both are spared, but not fully satisfied, by methionine.

Among several other micro-organism used for vitamin B_{12} assay, mention may be made of certain marine organism whose use has facilitated measurement of the vitamin in sea water.

Vitamin B_{12} occurs in nature in more or less stable complex forms and in some of these bound forms it may not be fully available for the test organism. Extraction procedures adopted to release the vitamin activity in free form vary in details under different circumstances. In general, use of certain enzyme preparations, mostly proteolytic, and/or heat treatment at pH 5 to 6 are resorted to for liberating the vitamin activity. During heat treatment presence of small amounts of cyanide or metabisulphite facilitates liberation of the activity.

All the organisms now in use for the assay of vitamin B_{12} respond to a greater or lesser extent to Factors A, B, C, pseudovitamin B_{12} and to other non-specific interfering materials. It is, therefore, extremely important to know their suitability for use under different conditions. Accuracy and specificity required, time, complexity of the assay technique would be among the important determining factors. Different microbiological methods used for vitamin B_{12} assay have been extensively and critically reviewed by different workers and it appears that the Chrysomonads respond to all naturally bound forms of vitamin B_{12} and like factors in a manner similar to that by animals, and offer, for the first time, a method specific for cobalamins. Nevertheless, the interfering materials may be essentially absent or present in low concentrations in certain materials, in which case other organisms can very well be employed.

Vitamin B_{12} can be assayed biologically by measuring the growth response of animals especially rats and chicks, previously rendered deficient. Difficulty is often encountered in satisfactorily depleting the test animals of their tissue stores of the vitamin and certain special procedures have to be adopted for preparing suitably pretreated animals. It is generally accepted that since biological assays can usually be carried out directly on a sample without submitting it to a rigorous extraction procedure they are a measure of the actual physiological availability of the vitamin. Moreover, the biological tests are not likely to be complicated by the presence of B_{12}-like factors and other interfering materials.

In view of imcompleteness of detailed information regarding the biochemical and nutritional aspect of the vitamin and considering the scope and limitations of different assay procedures, it is apparent that a correct idea about vitamin B_{12} activity of a substance can be had only from the combined results of several different assay techniques. However, for most of the common purposes where accuracy of a high order is not desired at the expense of time and complexity of the technique, any of the foregoing procedures can be adopted.

Research Notes

SULPHATE PRECURSOR BALANCE IN PENICILLIN PRODUCTION : A Preliminary Note

In their communications [1,2] on the sulphur metabolism of *Penicillium chyrsogenum* in synthetic medium, Tardrew and Johnson reported that (1) enchanced sulphate metabolism is characteristic of high yielding mutants of the mold; and (2) the labile biosynthetic precursor produced by such mutants can be successfully built up into penicillin only in the presence of adequate concentrations of the side chain (R-group) precursors in the fermentation broth.

To investigate the validity of these findings in the natural production medium containing cornsteep and lactose, shake flask experiments were carried out on a rotary shaker (280 r.p.m.) with a high yielding mutant strain of *P. chrysogenum* (HA-9) developed in this laboratory. Ten ml. of 48 hr. vegetative inoculum grown from spores in a cornsteep-sucrose medium were transferred to 500 ml. Erlenmeyer flasks containing 100 ml. of the production medium. The quantity of seed and concentration of spores were same in all experiments. Both phenylacetic acid and phenylacetamide were used as the R-group precursors. Additional doses of these were either incorporated in the medium initially or were added during fermentation as in experiment 6. Similarly, additional doses of sodium sulphate—the source of inorganic sulphur—were either added initially or fed during fermentation. Penicillin was assayed by the modified iodometric method.[3,4] The results are presented in the table below. The reading in each case is a mean of at least three replicates. Penicillin yield of the control with phenylacetic acid as the precursor is represented as 100.

Expt. No.	Total precursor	Total Na_2SO_4	Penicillin yield with precursor			
			Phenylacetic acid		Phenylacetamide	
	%	%	Readings	Mean	Readings	Mean
1	0.10	0.13	control	100	125 130 120 125	125
2	0.15	0.26	180 185 175 170	177.5	180 175 175 170	175
3	0.15	0.13 0.13 at 48 hrs.	150 145 140 145	145	165 165 160 150	160
4	*0.2	0.13 0.13 at 48 hrs.	180 175 175 170	175	180 170 180 170	175
5	0.2	0.39	160 155 155 150	155	155 165 160 160	160
6	0·2	0·13 0·13 at 48 hrs. 0·13 at 72 hrs.	180 175 170 175	175	175 175 175 175	175

* 0·1 initially and 0·1 at 48 hrs. of additional precursor fed as sodium phenylacetate.

As is evident from the table, there is a consistently higher yield of penicillin up to double the control, suggesting the existence of sulphate precursor balance for increased penicillin formation in the broth, thus confirming the findings of Tardrew and Johnson in synthetic medium. It appears that phenylacetamide is a better precursor than phenylacetic acid for strain HA-9. Further experiments with other strains and the behaviour of conditioned seed will be reported later.

REFERENCES

1. Tardrew, P. L., and Johnson, M. J. Sulfate utilization by penicillin producing mutants of *Penicillium chrysogenum*. *J. Bact.* **76**, 400, (1958)
2. *Idem.* Distribution of sulphur containing compounds in penicillin fermentation. *J. Biol. Chem.* **234**, 1850 (1959)
3. Alicino, J. F. Iodometric method for the assay of penicillin. *Industr. Eng. Chem.* (*Anal. ed.*) **78**, 619 (1946)
4. Mundell, M., Fischbach, H., and Eble, T. E. Chemical assay of penicillin. *J. Amer. Pharm. Assoc.* (*Sci. ed.*) **35**, 373 (1946)

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PHENOXYACETIC ACID*

Brettle[1] reported that the condensation of phenol and chloroacetic acid in aqueous alkali gave poor yields of phenoxyacetic acid and he prepared the latter in a two-step process through ethyl phenoxy-acetate in an overall yield of 56 per cent. In the course of our work on the preparation of penicillin precursors in 1956, we prepared the compound with a better yield, following a modified procedure of Koelsch[2].

EXPERIMENTAL

A solution of phenol (94 g. 1 mol.) sodium hydroxide (94 g.) dissolved in water (500 ml.) and freshly distilled chloroacetic acid (104 g., 1.1 mol.) was reflexed for 4 hr. on an oil bath. It was poured on a mixture of sufficient crushed ice and conc. hydrochloric acid (210 ml.). After about half an hour the precipitated solid (m.p. 74-86°) was filtered and washed with water. The crude material was crystallised from water (1500 ml.) yielding white needles of phenoxyacetic acid (104 g.), m.p. 99°. The mother liquor, after concentration to half the volume, gave a second crop (14 g.) m.p. 99-100°, increasing the total yield to 77 per cent of the theoretical.

REFERENCES

1. Brettle, R. Phenoxyacetic acid. *J. Chem. Soc.* 1891 (1956)
2. Koelsch, C. F. Identification of phenols. *J. Amer. Chem. Soc.* **53**, 304 (1931)

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* Since completion of our work in 1956 there has appeared a report of a similar work by Van Essen and M. G. J. Beers in *Recueil* **76**, 128 (1957)

A PRELIMINARY NOTE ON PHENOXY METHYL PENICILLIN ANALOGUES

As part of our general programme on the biosynthesis of acid stable penicillins, three new penicillin V analogues in which the aromatic ring of the side chain has been substituted in various positions by a methyl group, have been isolated. In order to avoid unknown factors in the fermentation a chemically defined medium of Jarvis and Johnson[1] was used. This medium was, however, slightly modified by adding the requisite amount of calcium carbonate to maintain the optimum pH plateau. The facility of incorporation of the precursor into the penicillin molecule and that of the isolation of corresponding penicillins was found to be greatest with *p*-methylphenoxyacetic acid followed by *m*-methylphenoxyacetic and *o*-methylphenoxyacetic acid in that order.

The melting points of some of the derivatives are as follows :

p-Methylpenicillin V	119-120°
Dibenzylethylenediamine salt of *o*-methylpenicilin V	110°

(corresponding salt of *o*-methylphenoxyacetic acid had m.p. 135°).

Further details of the work and stability studies on the new penicillins will be published elsewhere.

REFERENCES

1. Jarvis, F. G., and Johnson, M. J. Role of the constituents of synthetic media for penicillin production. *J. Amer. Chem. Soc.* **69**, 3010 (1947)

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Physico-chemical Data on Antibiotics*

I. ANTIBIOTICS PRODUCED BY FUNGI, BACTERIA AND LICHENS

2. **(a) Compounds for which empirical formula, melting point or u.v. absorption data, are not reported. (b) Index of antimicrobial activity.**

(a) The appended list supplements the data given in the first part, and the two parts together cover antibiotics from fungi, bacteria and lichens reported upto the end of 1958.

For the substances in the present list neither the empirical formula, the melting point, nor the absorption maxima have been reported.** The arrangement is alphabetical according to the producing organism, the serial numbering being continued from Part 1. To facilitate identification of the compounds, some of the reported physical, chemical data and antimicrobial activity are given.

(b) *Index of antimicrobial activity.* The tabular chart is a general indication of the antimicrobial activity of the compounds listed in Parts 1 and 2, providing approach to the substances by this biological property. The serial numbers correspond to the serial numbering in Parts 1 and 2. Abbreviations and symbols:

B : Antibiotic from bacteria

F : Antibiotic from fungi

L : Antibiotic from lichen

o : Weak or low antimicrobial activity

r : Limited range of activity against the particular type of organisms

x : Mainly active against the type of organism noted at the head of the column.

It is to be noted that no quantitative values of the antimicrobial activity are implied in the chart; only the general nature and range of activity are indicated. In a number of cases inhibitory activity is reported against one or two organisms only (indicated with "r") used as test. But this does not preclude activity of the compounds on other organisms in the same group, because the full range of activity of these compounds have not probably been investigated.

In addition to the general references mentioned in Part I. 1. the following publications have been helpful:

Carel, L., and Roach, E.S., *comp.* Dictionary of antibiosis. New York, Columbia University Press, 1951.

Shemyakin, M. M., and Kokhlov, A.S. Chemistry of antibiotics. Moscow, Goschmimizdam, 1953 (In Russian).

Literature on a number of antibiotics was also traced with *Chemical Abstracts* and *Biological Abstracts.*

A. NEELAMEGHAN

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* Pt. I. 1. *Hindustan Antibiot. Bull.* **2**: 13-38 (1959).

** Except in two cases where these data have been reported since publication of Pt. I, 1.

(a) **Physico-Chemical Data**

S. No. 1	Producing organism. Antibiotic 2	Physical-chemical characteristics 3	Active mainly against (microorganisms) 4
233	**Acetobacter acetigenum** Factor.		**Proteus vulgaris**
234	**Armillaria caligata** Factor.		**Staph. aureus**
235	**Armillaria mellea** Factor.		**Staph. aureus, E. coli (?)**
236	**Ascochyta Pisi** Ascochitin	Chitin containing substance	Sporogenous bacteria, gram—; weak against yeast.
237	**Aspergillus** sp. Mycocidin	Acidic. Forms water sol. and alk. insol. salts. Thermostable. Activity slightly reduced on incubation with human serum or Sorensen's phosphate buffer, pH 7.2-7.6.	**Myco tuberculosis** var. **hominis.**
238	**A. caespitosus.** Factor.	Dissolves in $NaHCO_3$ giving purple colour.	**Staph. aureus, C. diphtheriae, Myco. phlei**
239	**A. flavipes** Aspergillin		Gram+, gram−.
240	**A. flavus** Factor	Crystalline. Similar to Aspergillic acid (51).	Fungi
241	**A. fumigatus.** Factor		Lymphogranuloma cells. Carcinoma.
242	**A. humicola** Humicolin	Yellow oil. Weakly acidic. Contains no N, S or halogen. Brown colour with $FeCl_3$ in alc. soln. Unstable above pH 4·5. Inactivated by u.v. in $NaHCO_3$ soln. Not inactivated by cystein at pH. 3.5.	**Bact. globiforme;** partially **Staph. aureus, B subtilis.** High activity against fungi.
	A. niger Aspergillin (See 239)		
243	**A. parasiticus,** closely related spp. Aurantidin.	Orange red. Resinuous. Thermostable.	Gram+, gram− (Chloramphenicol type spectrum.)
244	**A. proliferans.** Proliferin	Cf. Variecolin (246)	Acid fast
245	**A. terreus** Factor		Gram+
246	**A. variecolor** Variecolin		**Myco. tuberculosis**
247	**A. velutinus** Velutinine		**C. albicans** and causative organisms of dermatomycoses
248	**A. wentii** Factor		(See **Chem. Abst. 51,** 2431a (1957)
249	**Bacillus** (aerobic, sporulating) Colistatin	Thermostable	Gram+, gram−

1	2	3	4
250	**Bacillus sp.** Factor		**Haemophilus pertussis**
251	**B. abysseus** Factor	Not inactivated by Seitz and Mandler filtration	Gram+
252	**B. Alvei** Alvein	Probably strong basic polypeptide. Thermostable at acid pH, less so at alkaline. Inactivated by trypsin. C 49.0, H 8.7, N 12.9, S 1.2, Cl 8.9. Arginine, cysteine, lysine, serine, threonine, alanine, valine, leucine in hydrolysate.	Gram+, acid fast
253	**B. borborokoites** Factor	Not inactivated by Seitz or Mandler filtration	Gram+
254	**B. brevis-like organism** Factor		Gram—
255	**B. brevis** Brevin.	Contains 8.54% N, aspartic acid, tyrosine, serine, lysine and a basic, ninhydrin blue substance. Purified Brevin gives u.v. max. at 275 mu (18% tyrosine) in 75% EtOH, pH 3.0	Gram+, **Myco. tuberculosis**
256	**B. brevis** Gramicidin J	Cyclic heptapeptide containing D- and L- phenylalanine, D-leucine, D- and L-ornithine, L-valine, L-proline. 292-93° dec. $(\alpha)_D$ —308.9 (EtOH, 18°). λ_{max} 250, 258, 265 mμ (MeOH). C 58.12, H 8.03, N 13.7, cl 5.4.	Gram +
257	**B. brevis** Ovothricin		Gram+
258	**B. brevis** Tyrothricin	Polypeptide mixture of 20% Gramicidin (175) and 80% Tyrocidine (181)	Gram+, less active against certain gram—, fungi spirochaetes, protozoa
259	**B. cereus** Biocerein	Brownish yellow oil. Activity decreases in presence of rabbit serum.	Gram+, gram—
260	**B. cereus** (α strain) Cerein B_2	Colourless, amorphous. Micromolecular polypeptide. Readily dialyzable. Rf 0.7 (BuOH-formic acid-water). Dec. by acid and base. No loss on lyophilization and vacuum evaporation. Aq. soln. thermostable. 0.01N NaOH antagonizes activity. Resistant to trypsin.	Gram—
261	**B. cereus** Factor		Lyses dead cells of bacteria
262	**B. circulans** Lytic enzyme	Optimum activity at pH 6.8. On lysis of cell walls of fungi a polymer (probably of melibiose) is liberated. Glucose and galactose in hydrolysate.	Lyses cell walls of fungi, heat treated **Saccharomyces sake** but not of **Rhizopus javanicus**
263	**B. colistinus** Colimycin	Cyclic polypeptide of Polymyxin group. Threonine, leucine, α, γ-aminobutyric acid in hydrolysate.	Gram+, gram—
264	**B. colistinus, B. polymyxa** Colistin	Peptide. Leucine, threonine, γ-diamino-butyric acid, D-6-methyloctanoic acid in acid hydrolysates. Inactivated by HNO_2, $BzCl_2$, Ac_2O.	**Haemophilus pertussis**
265	**B. larvae** Factor	Adsorbed on charcoal but cannot be eluted. Not dialyzable.	Gram+, gram—, acid fast
266	**B. laterosporus** Laterosporins A, B	A—sol. in EtOH, B—insol. in EtOH. Polypeptides. Dialyse. Horse serum reduced activity.	Gram+, gram, acid fast.

1	2	3	4
267	**B. megatherium** Megacine	Slightly diffusible in agar. Does not pass through parchment. Moderately thermostable. Pptd. by $(NH_4)_2SO_4$ at 75% saturation. Trypsin inactivates.	**B. megatherium**
268	**B. mesentericus** Factor 1	Stable to heat, freezing, desiccation, acid. Alkali labile. Withstands autoclaving for a short time.	Causes abnormal hyphal growth, inhibits germination and induces mutations in some races of **Helminthosporium sativum**
269	**B. mesentericus** Factor 2	Thermolabile. Solid substance by chromatography.	Gram+, gram—
270	**B. mesentericus vulgatus** Acids	Extract contains HCOOH, AcOH, EtCOOH, PrCOOH, stearic, oleic, elaidic acids,	Oleic acid toxic to **Staph. aureus, C. diphtheriae.** Elaidic acid toxic to **C. diphtheriae** only
271	**B. niger** Nigrin		Gram+
272	**B. prodigiosus** Factor		**S. violaceus, S. aurantiacus, S. griseus, S. globisporus**
273	**B. prodigiosus** (pigmented strain) Factor		Gram +
274	**B. proteus** Protaptin		Acid fast
275	**B. pumilus** Tetaine	Colourless, amorphous, hygroscopic powder. Positive ninhydrin. Negative for COOH, NH_2. Dialyzable. No. ppt. with protein precipitants. Aq. soln. stable upto boiling point, unstable on drying. Slightly inactivated by serum, resistant to trypsin.	Gram+, gram—
276	**B. simplex** Simplexin	Acid stable, alkali labile.	Gram+, gram— **Rhizoctonia solani**
277	**B. submarinus** Factor	Activity not lost on Seitz or Mandler filtration.	Gram+
278	**Bacillus** sp. probably **B. subtilis** Petrin	Contains no N. Adsorbed on charcoal but not readily eluted. Boiling at pH 8 reduces activity by 66 % in 10 min., remaining constant thereafter. Stable at room temp. at pH 4.5 to 11. Stable to trypsin, pepsin and dry heat.	Gram—
279	**Bacillus** sp. resembling **B. subtilis** Factor	Thermostable.	Gram+
280	**B. subtilis** Aspergillus factors, I, II	I—Viscous brown; II—greyish white powder. Do not diffuse through cellophane membrane. Stable at 100° for 15 min. at pH. 2.5. II loses some activity at pH. 0.5-1.5.	Fungi
281	**B. subtilis** Bacilysin	Neutral peptide containing S. Positive ninhydrin, Pauly. Acid hydrolysates contain alanine, tyrosine, phenylalanine, leucine. No ppt. with $(NH_4)_2SO_4$, basic lead acetate, picric, phosphotungstic, trichloroacetic acids. Thermostable except at low pH. Inactivated by H_2S, enzymes, trypsin.	Gram+; low activity against acid fast.

1	2	3	4
282	**B. subtilis** Bacillin	No ppt. with Hg, Ag, Pb salts, phosphotungstic, 2, 4-dinitrophenylhydrazine and other acids. Thermostable. EtOH does not inactivate. Inactivated by blood, H_2S and some media.	Gram +, gram—, **Myco. tuberculosis**
283	**B. subtilis** Bacilipins A, B	Unsaturated aliphatic acids. Negative Molisch, 2, 4-dinitrophenylhydrazine, ammoniacal $AgNO_3$. Salts thermostable. Analysis, Ba salt of A: C 42.6, H 6.3, N 2.5, Ba 24 6; Ba salt of B: C 52.5, H 6.75, N 2.09, Ba 21.6. Not digested by trypsin resistant to serum.	Gram+, **Myco. phlei.** Low activity against gram—
284	**B. subtilis** Bacillomycin B.	Yellow, amorphous. Polypeptide. Isoelectric point. pH 4.3-4.5. No S or halogen. Positive biuret, xanthoproteic, Millon, Liebermann. Negative ninhydrin, $FeCl_3$, Molisch. Red brown with conc. H_2SO_4, red on heating. No reduction of Fehling, Tollens. Contains glutamic acid, aspartic acid, tyrosine, leucine, proline. Non-dialyzable. Thermostable at pH 2.6-8.2.	Fungi.
285	**B. subtilis** Bacillomycin C	Polypeptide. Isoelectric point, pH 4.2. Positive biuret, xanthoproteic, Millon, Liebermann. Negative ninhydrin, $FeCl_3$, Mollisch, Fehlings, Tollens. Red colour with hot H_2SO_4. Contains glutamic acid, tyrosine, leucine, valine; no threonine, serine, proline. Fairly thermostable.	
286	**B. subtilis** Endosubtilysin.	Stable at 60°, best stored in cold.	**Staph. aureus**
287	**B. subtilis** Eumycin	Colourless acid. Resembles Bacillomycin. Thermostable, unstable in alkaline soln. above pH 8.	**C. diphtheriae**, acid fast fungi.
288	**B. subtilis** Fungistatin (Antibiotic XG)	Amphoteric polypeptide. Gels on standing in water losing activity, reactivated by dissolving in anhydrous solvents. Contains lysine, serine, aspartic acid, proline, threonine, alanine, tyrosine, tryptophan, valine, isoleucine, etc. Heat stable at acid and neutral pH.	Fungi.
289	**B. subtillis** Globicin	Ppt. with $(NH_2)_2SO_4$. No colour with $FeCl_3$. Contains tryptophan and loosely bound S. Adsorbed on asbestos pad filters. Thermostable at pH 2-8.5. Albumin V reduces activity.	Gram+, acid fast
290	**B. subtilis** Factor (1953)	Yellow substance	Gram—
291	**B. subtilis** Mycobacillin	Light brown needles. Cyclic polypeptide. Positive Millon, biuret, Folins, phenol, xanthoproteic. Negative ninhydrin, Molisch. N content 11.9%; No S, halogens. Hydrolysate contains aspartic acid, glutamic acid, serine, alanine, tyrosine, leucine, proline.	Fungi
292	**B. subtilis** Neocidin	Light yellow powder. Light sensitive. Negative biuret, Ehrlich aldehyde. 1% aq. soln. faintly opalescent. Thermolabile, stable at pH 5-6.4.	Staphylococci, **B. anthracis**, **B. subtilis**, **Myco. avium**
293	**B. subtilis** Obutin	Similar to Neocidin.	Staphylococci, **B. anthracis**
294	**B. subtilis** Neopumilin		Grams+

1	2	3	4
295	**B. subtilis** Rhizoctonia Factor	Diffuses slowly through cellophane membrane. Thermostable at pH 2.5.	Fungi.
296	**B. subtilis** Subtilin	Amorphous white powder, Basic polypeptide, $(\alpha)_D$ -29 to -35 (acetic acid, 23°). Dialyzable. Blue with $FeCl_3$. Salt decreases solubility in acid soln. Contains glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, lysine, asparagine, glutamic acid, lanthionine, $C_7H_{12}N_2O_3S$, amide. Inactivated by light, alkali, pepsin.	Gram+, **N. gonorrhoeae**, protozoa, virus. **Rickettsia tsutsugamushi** in chick embryo
297	**B. subtilis** Subtilin C	Powder. Polypeptide. Diffuses slowly. Positive ninhydrin, Ehrlich, Folin-Denis. Negative $FeCl_3$. Activity destroyed by sunlight and alkali. Most stable at pH 2.5. No loss on Seitz filtration, with serum during 24 hrs. and amino acids and vitamins.	Acid fast. Weak against gram+
298	**B. subtilis** Subtylysin	Powder. Resembles Actinomycetin. Pptd. from aq. soln. by 10% $CaCl_3$. Aq. soln. moderately thermostable.	Gram+, gram—
299	**B. subtilis** Trypanotoxin	Destroyed on heating at 73° for 20 min.	Certain rickettsiae.
300	**B. subtilis** Xanthellin	Na salt: Pale yellow amorphous solid. Non-hygroscopic. Acidic. Dialyzable. Pptd. from aq. soln. on acidification. Thermostable, unstable at room temp. except at neutrality. Activity decreased by hog serum	Gram+, gram—, moderately against acid fast
301	**B. subtilis** Toximycin	Polypeptide or protein. Positive Millon, xanthoproteic, biuret, ninhydrin. Negative Fehling. Slowly dialyzable. No inactivation by pepsin or trypsin. Thermostable in buffer pH. 6.8, thermolabile in water esp. at alkaline pH. No loss on autoclaving.	Fungi. Weak against gram+
302	**B. thalassokoites** Factor	Activity not lost on Seitz or Mandler filtration	Gram+
303	**Basidiomycete** sp. Corticin		Gram+
304	**Basidiomycete** sp. Grisic acid		
305	**Basidiomycete** sp. Irpexin		
306	**Basidiomycete** sp. Obtusin		
307	**Basidiomycete** sp. Pleurin		
308	**Boletus edulis** Factor	Light tan coloured. Acid stable for a short period. Contains peptides of low molecular wt. Alanine, arginine, aspartic acid, glycine, glutamic acid, lysine, threonine, valine, leucine and 3 other ninhydrin, blue spots on chromatogram.	Crocker mouse sarcoma 180 in vivo.
309	**Camosporium** sp. Factor.	Deep red substance	**Sarcina citrea**, fungi

1	2	3	4
311	**Clitopilus abortivus** Factor		Tumour
312	**Collybia radicata** Factor		Tumour
313	**Coniophora cerebella** Factor 1		Koch bacillus, acid fast
314	**Coniophora cerebella** Factor 2		Gram+, gram—
315	**Coprinus picaceus** Picacic acid	Pale yellow oil (crude form). Stable at pH 2-7 room temp., inactivated at pH 9. Activity reduced on incubation with horse serum.	Gram+. Weak against gram—
316	**Corynebacterium diphtheriae gravis** Factor		Gram+, gram—
317	**Diplococcus** X5 Diplomycin		Gram+, **Myco. tuberculosis**
318	**Dothichiza populea** Toxin.	Activity decreases with rise in pH	**B. subtilis, Ustilago** spp.
319	**Escherichia coli** Colicines	Amorphous powder, odourless, gelatine-like taste. Polypeptides, proteinic. Thermostable in acid or neutral. Dialyzable. Pptd. by neutral acids from aq. soln. Pepsin, trypsin, liver tissue destroy activity.	Gram+, **Mycophlei**
320	**Escherichia coli.** Colicine K.	Colourless. Composed of carbohydrate, protein, lipid. N 6.5, P 1.6, no nucleic acid. Destroyed by trypsin, chymotrypsin, formalin.	**E. coli.**
321	**Escherichia coli** Factor		**Myco. tuberculosis** var. **hominis**
322	**Escherichia coli** Factor		Fungi
323	**Fusarium** sp. Chlamydosporins A, B.	A—Light brown, amorphous. B—Colourless cryst. A and B contain 4.3 % N but no S. Stable to autoclaving. Resistant to peptone, slight inhibition by serum.	Gram+, gram—, **Myco. tuberculosis.**
324	**Fusarium oxysporum** Fusanin A.	EtOH and acetone soluble. Inhibited by cysteine.	Gram+
325	**Fusarium oxysporum.** Fusanin B	Inhibited by cysteine.	Gram+, gram—
326	**Fusarium oxysporum** Oxysporin		**Myco. tuberculosis** var. **hominis**
327	**Gloeosporium olivarum** Factor	Antibiotic produced in agar media in presence of 2, 4-D.	Gram+, gram—, acid fast, **Gloeosporium olivarum.**
328	**Inoloma traganum.** Inolomin	Yellow, glassy, hygroscopic, amorphous. Thermolabile, stable at room temp. Darkens losing activity on autoclaving. Seitz filtration does not affect activity.	Gram+, specifically **Micrococci, pseudodiphtheriae.**
329	**Irpex destruens** Destruin	Yellow oil. Contains C, H, O. Stable at pH 2-7 at room temp., slowly inactivated at pH. 9. Serum lowers activity.	Gram+

1	2	3	4
330	**Lactarius** spp. Factors I, II	I—Thermolabile. II—Thermostable	Gram+
331	**Lactobacillus** sp. resembling **L. helveticus** Factor		**Staph. aureus**
332	**Lactobacillus acidophilus.** Factor	Lytic action depends on dilution in whey broth	Lyses cells of **Sarcina flava**
333	Leiwan strain, Factor		Protease action, vermifuge against tape worm of frog.
334	**Lenzites thermophila.** Thermophillin	Golden plates. Quinonoid. Volatalises at 245°, dec. at 260° heated in sealed tube. C_{18} H_{18} O_9. Aq. soln. yellow, changing to red with NaOH. Liberates I from KI, reduced to colourless compound by Na hydrosulphite. Bright red colour with conc. H_2SO_4 becoming brown on heating and yellow on addition of EtOH.	Weak against **Staph. aureus**
335	Lichen sp. Evosine		Gram+, acid fast
336	**Lycogala epidendrum.** Factor		Gram+
337	**Lycogala flavofuscum.** Factors I, II, III	Thermostable. Water insol. fraction is brownish red, deliquiscent amorphous powder. In C_6H_6 u. v. bands at 444-460 mμ (max. 482 mμ), 507-523 mμ. C_6H_6 soln. shows birefringence.	I-**Mucor** spp., **Lycogala flavofuscum.** II, III. **Strep. putridus**
338	**Malleomyces pseudomallei** Whitmorin	Thermolabile, unstable at 37°.	Gram+, **Neisseriae, Myco. tuberculosis** var. **hominis**
339	**Marasmius ramealis.** Factors I, II	I. Non-volatile. II. Volatile. I. Stable in dry or in soln. II. Decolourises $KMnO_4$ soln. and Br water. Ppt. with $HgCl_3$ soln. Change into inactive substances at room temp.	Gram+, gram—
340	**Marasmius urens.** Factor		**Staph. aureus**
341	**Micrococcus** sp. Factor		**Brucella** spp.
342	**Micrococcus** spp. Factor		**S. violaceus, S. aurantiacus**
343	**Micrococcus infimus** Factor		**Sarcina lutea**
344	**Micrococcus maripuniceus** Factor.		Gram+, **Myco. lacticola**
345	**Micrococcus pyogenes** var **albus.** Factor		Gram+
346	**Micrococcus sedimenteus** Factor		**Sarcina lutea**
347	**Micrococcus tetragenus** Trevisan var. Trevisin	Stable at refrigator temp., and heating at 60° for 15 min. Activity reduced at higher temp. Most active at pH. 7.5	Gram+, **Myco. avium** weak against gram—

1	2	3	4
348	**Microsporum canis** Factor		**Staph. aureus**
349	**Monilia antipiricularia** Antiblastin		Fungi
350	**Monilia antipiricularis.** Antiburastin		**Piricularia oryzae, Ophiobolus miyabianus**
351	**Mucilago spongiosa.** Factor	Saponification with dil. H_2SO_4 gives an unsaponifiable factor active against **Strep. putridus** only, unsaponified factor is active against **Cl. septicum** also	**Strep. putridus, Cl. septicum**
352	**Mucor racemosus** (minus strain). Factor	Thermostable	**Sarcina lutea, Serratia marcescens**
353	**Mycobacterium** sp. (disrupted cells). Factor	Colourless needles. Rapidly loses potency	**Myco. avium**
354	**Mycobacterium** spp. Factor		**S. violaceus, S. aurantiacus.**
355	**Mycococcus** spp. Factor		**S. violaceus, S. aurantiacus**
356	**Mycoccus citreus, lactis, luteus.** Factor		**S. violaceus, S. aurantiacus S. griseus, S. globisporus**
357	**Nostoc muscorum** Factor	Extractable with organic solvents, not miscible with water.	Gram+, yeast, algae
358	**Oospora sulphureaochracea** Sulphochracein	Impure white powder. Aq. soln. stable at pH 7-8 at room temp., but inactivated at pH 5.5 and below. Inactivated by cystein.	Weak against gram+
359	**Oospora virescens** Factor		Fungi
360	**Pasteurella pestis** Pesticin	Bacteriocin-like substance. Sensitive to proteolytic enzymes. Diffuses slowly through agar and does not pass through cellophan. Thermolabile. Stable between pH 6 and 8. Resistant to u. v. irradiation. Adsorbed by Seitz filters and partly by sintered glass filters.	**Pasteurella pseudotuberculosis.**
361	**Penicillium** sp. Factor		**Salmonella typhosa**, tadpoles
362	**Penicillium** sp. Factor		**Venturia inequalis**
363	**Penicillium** sp. resembling **Citromyces pferferronionus.** Factor A, B	A—White needles.	Gram+
364	**P. albidum.** Factor	Colourless cryst. Contains no N, S or halogen. Deep green colour with $FeCl_3$	**Botrytis allii**
365	**P. carneolutescens.** Carneolutescin	Oil containing C, H, O. Negative $FeCl_3$, Fehling. Stable at room temp. at acid pH, less stable at higher pH. Serum destroys activity.	Gram+, **Salmonella enteridis**

1	2	3	4
366	**P. chrysogenum.** Trichocidin	Organism cultured in CZapek-Dox at 20°	Protozoa
367	**P. cyaneofulvum.** Noxiversin	Consists of a homogeneous carbohydrate fraction and a heterogeneous protein fraction.	Inactivates bacterial toxins
368	**P. divergens.** Factor		Gram+, gram—
369	**P. funiculosum.** Helenine	Stable when frozen. Thermostable. Pptd. from 50% acetone. Non-dialyzable but filters through Seitz pad. Acetone pptd. material contains N (total Kjeldahl) 1.29, P 0.26, P as free phosphate 0.2, P (bound) 0.06. Polysaccharides 52. Reducing sugars about 7·0.	Viruses
370	**P. lilacinum.** Factor		Gram+
371	**P. notatum** Factor	Acetate sol. in common solvents. C 70.61, H 4.56. Iodine no. about 160. About 7% N and at least 1 phenolic OH group. Forms acids, alkali metal and alk. earth salts.	Bacteria
372	**P. notatum** (non pigmented strain). Antibiotic X29c.	Yellow. Does not contain S, N, halogens.	Gram+, gram—
373	**P. notatum** Notalysin		Gram+, gram—
374	**P. pulvillorum** Pulvilloric acid	Buff coloured needles turning bright yellow in air or in vacuo over P_2O_5 in light or darkness. Acid. Colourless soln. in MeOH, EtOH, BuOH, bright yellow in acetone, ether, dioxan. Liberates CO_2 from $NaHCO_3$ soln. Alc. soln. gives blue colour with $FeCl_3$. Tollens not reduced. Contains no N, S or halogens. Aq. soln. stable at 25° at pH 3-8.	Fungi
375	**P. reticulosum** Factor	Crude substance pale brownish yellow. Positive Molisch. Ash content 3.8%. 1% soln. in 0.1N HCl inactivated overnight at room temp.	**Staph. aureus**
376	**P. spinulosum** Factor	White fine needles.	Gram+, gram—
377	**P. velutinum** Factor		Synergistic action with Citrinin
378	**Pfefferella whitmori** Whitmorin	Fairly heat stable, withstands brief boiling. Activity retained on refrigeration but lost on storing at 37°.	Gram+, gram—, **Myco. tuberculosis var hominis**
379	**Phycomyces** sp. (Phytophthora or Pythium) Factor		**Trypanosoma equiperdum**
380	**Pleurotus mutilis** Factor		Gram+, gram—
381	**Polyporus cinnabarinus** Factor	Hygroscopic. Stable at room temp. Thermostable.	Gram+
382	**Polystictus sanguineus** Polyporin	Acidic. Non volatile, thermostable at 120° for 20 min. but not in acid and alkali. Not affected by HCl, pepsin, gastric juice, blood, serum, etc.	Gram+, gram—

1	2	3	4
383	**Polystictus versicolor.** Polystictin	Yellow, hygroscopic powder. Dialyzes through cellophane. Not extracted from aq. soln. by phenol and not pptd. by heavy metal ions and basic precipitants. Stable at pH 2-10 for 3 hr. in aq. soln. and on boiling at pH 2. Destroyed on boiling at pH. 7-10. Not inactivated by pepsin or trypsin.	**Staph. aureus, E. coli** (?)
384	**Psalliota campestris.** Campestrin		Gram+, gram—
385	**Psalliota xanthoderma** Psalliotin	Stable at room temp. Loses activity on exposure to daylight or short u.v. or daylight passing the deep violet wrelten filter D. Uneffected by yellow light from a Philips dark room globe.	Gram+ cocci, gram— intestinal pathogens
386	**Pseudomonas aeruginosa.** Pioklastin		**Neisseriae gonorrhoeae**
387	**Pseudomonas pyocyanea** Pyocine		**Pseudomonas pyocyanea** 13
388	**Reindeer lichen.** Usno	Tetracycline type.	
389	**Rhizopus nigricans.** Factor		Gram+, gram— (doubtful results)
390	**Rhodotorula glutinis** var **lusitanica.** Lusomycin	Carotenoid, similar to Karrer and Rutschmann's torularhodine.	(*See* **Chem. Abst. 48,** 13807a, 1954)
391	**Rhodotorula suganii** Factor		Acid fast
392	**Sclerotinia sclerotiorum.** Factor		**Staph. aureus,** Wheatley streptococcus
393	**Serratia marcescens** Factor	Heat stable in broth. Dialyzable. Not a lipoid, neutral fat or fatty acid.	Gram+, gonococci
394	**Staphylococcus aureus** Factor	Thermostable in neutral and acid pH. Above pH 5 activity destroyed at room temp. Also destroyed by trypsin and pepsin.	Gram+, **Myco. phlei**
395	**Stemonitis fusca** Factor		**Strep. putridus,** and **Cl. septicum** on warming the extract.
396	**Stereum rameale** Ramealin	Pale yellow oil. Na and Ba salts hygroscopic, sol. in water. Decolorises alk. $KMnO_4$ in cold, colour with $FeCl_3$. C 72.4, H 7.6, CH_3 (C) 7.35, no alkoxyl. Unstable in alkali. Incubation with horse serum reduces activity.	Weak activity against **Staph. aureus**
397	**Sterigmatocystis.** (Aspergillus) Sterigmatocystin	Activity not lost at 60° for 5 min. but reduced at 80°	**Staph. aureus**
398	**Streptococcus faecalis** Factor		**Proteus mirabilis**
399	**Streptococcus haemolyticus** (β group) Streptostasin	Diffuses through agar. Not neutralised by sera.	**Streptococcii**

1	2	3	4
400	**Streptococcus lactis.** Diplococcin	Protein-like. Lavorotatory. Negative Millon, Heller, Molish. Pptd. by half saturated $(NH_4)_2SO_4$ slow diffusion through colloidon. Positive for argnine, tyrosine, tryptophan. Thermostable in acid, labile in alk. soln.	Gram+
401	**Streptococcus liquefaciens** Factor		**Proteus mirabilis**
402	**Streptococcus mitis** Factor	Antibiotic activity probably due to production of H_2O_2.	**Corynebacterium diphtheriae**
403	**Streptococcus pyogenes** Factor		Gram—
404	**Tea fungus.** Factor		**Bact. pyocyaneus.**
405	**Trichoderma** sp. Factor	Volatile	Bacteria, fungi.
406	**Trychophyton gypseum** Trichomycin		Gram+
407	**Trychophyton rubrum.** Phytorubin	Antibiotic from mycelium	Gram+, **Myco. tuberculosis**, yeast-like fungi.
408	**Trichophyton rubrum**	Produced with Phytorubin (407)	More active than Phytorubin but less active than whole product
409	**Trichothecium** sp.		**Plasmopara viticola**
410	**Tubifera feruginosa** Factor		**Clostridium histolyticum**
411	**Usnea acciculiphera** Factor		**Lactobacillus plantarum**
412	**Ustilago avenae, Ustilago** spp. Ustizeains A,B.	Stable, fat soluble substances	Gram+, gram—, fungi
413	**Volutellospora cinnamonea** Factor		**Myrothecium verrucaria**
414	Yeast Factor	Watery extract. Isoelectric point about pH 2.0. Contains some protein.	Gram+, gram—, fungi
415	Yeast Malucidin		Gram+, gram—, fungi
416	Yeast Y_1, Y_2, other factors.	Colourless compounds. Chromatogram gives negative ninhydrin, diazotised sulphanilic acid, Ehrlich, cyanogen bromide. Picric acid and 2, 4-dinitrofluorobenzene derivatives not formed. Y_1, acid hydrolysate contains leucine, valine, alanine, glutamic acid, glycine. Y_2 contains these and γ-aminobutyric acid. U. V. max. for Y_2 at 255 mμ. I. R.for Y_1, Y_2 3.0, 3.4. 5.95, 6.1 (Y_1) or 6.2 (Y_2), 6.9-7.0, 8.0 (Y_2) μ.	**M. pyogenes** var. **aureus, A. niger, P. glaucum**

(b) INDEX OF ANTIMICROBIAL ACTIVITY

*S. No.	Gram+	Gram−	Acid fast	Fungi and Yeast	Actinomycete	Protozoa	Rickettsiae	Spirochaete	Virus	Phage	Tumour	Others
1	2	3	4	5	6	7	8	9	10	11	12	13
1F	o	o		x								
2BF	x	x	x									
3B	x	x										
4BF	o	o										
5F	x	o	x						x	x		
6F	x	x									x	
7F	x	x		x		x						
8F	o											
9F	1											
10F	x	x										
11F	x	x		x								
12F	x											
13F				x								
14F	x											
15F				x								
16F	x	x	x									
17F	x	x										
18F	x	o		o					x			
19F	x	o	o									
20B	x		x									
21F	x	x		x								
22F				2								
23F	3											
24F	x	o										
25BF	x	x	x									
26F	r	r										
27F				x								
28F	r			r								
29F	r											
30F				r								
31F			x							x		
32F	r			r								
33F				o								
34F	o	o										
35F	r		r									
36F	o	o										
37B	x	x										
38F	r											
39F	x	x		x								
40B	x	x										
41F	x	x		o								
42F				x								
43F				r								
44F	x	x		x								
45F	x			x								
46B			x									
47B	x	x		x								
48B	x											
49B	x	x		x								
50F	o	x										
51F	x	x	x									
52F	o	o										
53F	x	x	x	x						x		
54B	x	x		x								
55B	r											

* See *Hindustan Antibiotics Bulletin* **2**: 18-38 (1959)
(1) Acivitity due to contamination with diatretyne 1 (S. No. 12)
(2) *Zygosaccharomyces sulsus* (3) Sake putrefying bacteria.

1	2	3	4	5	6	7	8	9	10	11	12	13
56F	x	x		x							x	
57B	r			x								
58F	x											
59F	x		x									
60F				x								
61F	r	r										
62F	r											
63F	x	r						x				
64F	x	x										
65F	o	o		x								
66F	x	x	o									
67F												
68L	r		x									
69F			x									
70F			x									
71F	x		x									
72F	x		x									
73F	o	o		o								
74F	x	o										
75F	x	o	x	x								
76F	x	x										
77F			x	o								
78F	x		x									
79F												
80L	r		x									
81FL	r											
82F	r		x									
83F	x		x									
84F	x	r						x				
85F	x	r						x				
86F	x	r						x				
87F	x	r						x				
88B												
88aF	r	r										
89B												
90F	x	r						x				
91F	x	o	o									
92F	x		x									
93F	x	x	x									
94F	o	o		x								
95L			x									
96F				x								
97F				x								
98F				r								
99F	x			x								
100B	x	o										
101L			x									
102F	x	x	x									
103L	r											
104F	x		x									
105L	r											
106L	r											
107L	x	x	x	x								
108F												
109L	r											
110B												
111B	x	x										
112F	o	o										
113F			x									
114L			x									
115F	x		x									
116F				x		o						
117F				x		o						
118L	r											
119L	r											
120F	x	x	x									

1	2	3	4	5	6	7	8	9	10	11	12	13
121F				x								
122F	r		x									
123L	r		x									
124L	r											
125L	r											
126L	r											
127F	x	o	x									
128L	r											
129B				x		x						
130F	o	o		r								
131F	r	o	o									
132F	r		x									
133F	o	o										
134L	r		x									
135F				x								
136L	r											
137L	r											
138L	r											
139L	r		x									
140L	r		x									
141F	x											
142F	x	o	x									
143F			x	r					x			
144F	r											
145L	r											
146L	r											
147F				x								
148F	x		x	x								
149F	x		x									
150F	r			x								
151B	x	r	x									
152L	r											
153L	r		x									
154L	r											
155F	x		x									
156L			x									
157L	r		x									
158F					x				x			
159F	x		x									
160F	x		x									
161B	x	x		x								
162F	x	x	x	r								
163L	r											
164F	o	o	x									
165F	x	x	x	x								
166F	x		x									
167B			x									
168B	x	r		x		x						
169F	x	x	x		x							
170B	o	x		o		o						
171F				x								
172B		x										
173B	x	x	x	x								
174B		x										
175B	x	x										
176B	x	o			x	o						
177B												
178B	x											
179B	x	o			x	o						
180B	x											
181B	x	o			x	o						
182B	x				x							
183B												
184F	x	x	x	x					x			
185F		o										
186F	r		x									

1	2	3	4	5	6	7	8	9	10	11	12	13
187F	r		x									
188F												
189F	x	o										
190B	x	r	x									
191B	x					x						
192B	x					x						
193B	x	x										
194B	x	x	o									
195B			x									
196B	x											
197B	x	o										
198B												
199B												
200B												
201B												
202B	x			r								
203B												
204B												
205B	x	r										
206B			r									
207F												
208F												
209F			x									
210F	x	x	x									
211F												
212B	x	x	x									
213F	x	x	x									
214F	r											
215F	x	x	x									
216B				x								
217F		r										
218F			x									
219F	x	x										
220F	x		x									
221F	o	o		x								
222F	r	r										
223F	x	x										
224F	x	x										
225F			r									
226F	x	x	x	x								
227F	x	x	x	x								
228B				x								
229B			x									
230B	x	x	x									
231F	r											
232B	x	x	x		x							
233B		r										
234F	r											
235F	r											
236F	r	x		o								
237F			x									
238F	r		x									
239F	x	x										
240F				x								
241F												
242F	r			x							x	
243F	x	x										
244F			x									
245F	x											
246F			x									
247F				x								
248F												
249B	x	x										
250B		r										
251B	x											
252B	x		x									

1	2	3	4	5	6	7	8	9	10	11	12	13
253B	x											
254B		x										
255B	x		x									
256B	x											
257B	x											
258B	x	o		o		o		o				
259B	x	x										
260B		x										
261B	x	x										
262B				r								
263B	x	x										
264B		r										
265B	x	x	x									
266B	x	x	x									
267B	r											
268B				r								
269B	x	x										
270B	r											
271B	x											
272B					x							
273B	x											
274B			x									
275B	x	x										
276B	x	x		r								
277B	x											
278B		x										
279B	x											
280B				x								
281B	x		o									
282B	x	x	x									
283B	x	o	x									
284B				x								
285B												
286B	r											
287B	r		x	x								
288B				x								
289B	x		x									
290B		x										
291B				x								
292B	x		x									
293B	r											
294B	x											
295B				x								
296B	x	r				x	r		x			
297B	o		x									
298B	x	x										
299B												
300B	x	x	o									
301B	o			x								
302B	x	x										
303F												
304F												
305F												
306F												
307F												
308F												
309F	r			x						x		
311F												
312F										x		
313F		r	x							x		
314F	x	x										
315F	x	o										
316B	x	x										
317B	x		x									
318F	r			r								

1	2	3	4	5	6	7	8	9	10	11	12	13
319B	x		x									
320B		r										
321B			x									
322B				x								
323F	x	x	x									
324F	x											
325F	x	x										
326F			x									
327F	x	x	x	r								
328F	x											
329F	x											
330F	x											
331B	r											
332B	r											
333F												1
334F	r											
335L	x		x									
336F	x											
337F	r			r								
338B	x	r	x									
339F	x	x										
340F	r											
341B		r										
342B					x							
343B	r											
344B	x		r									
345B	x											
346B	r											
347B	x	o	x									
348F	r											
349F				x								
350F				r								
351F	r											
352F	r	r										
353B			x									
354B					r							
355B					r							
356B					x							
357F	x			x								2
358F	o											
359F				x								
360B		4										
361F		r										3
362F				r								
363F	x											
364F				r								
365F	x	r										
366F						x						
367F	x	x										
368F	x	x										
369F									x			
370F	x											
371F	x	x										
372F	x	x										
373F	x	x										
374F				x								
375F	r											
376F	x	x										
377F												
378F	x	x	x									
379F						r						
380F	x	x										

(1) Vermifuge against tapeworm of frogs.
(2) Algae. (3) Tadpoles. (4) *Pasteurella pseudotuberculosis.*

1	2	3	4	5	6	7	8	9	10	11	12	13
381F	x											
382F	x	x										
383F	r											
384F	x	x										
385F	r	r										
386B		r										
387B		r										
388L												
389F	x	x										
390F												
391F			x									
392F	r											
393B	x	r										
394B	x		x									
395F	r											
396F	o											
397F	r											
398B		r										
399B	r											
400B	x											
401B		r										
402B	r											
403B		r										
404F		r										
405F	x	x		x								
406F	x											
407F	x		x	x								
408F	x		x	x								
409F				r								
410F	r											
411L	r											
412F	x	x		x								
413F				r								
414F	x	x		x								
415F	x	x		x								
416F	x			r								

Naturally, now that DET, Swastik's modern detergent, makes your clothes a dream of cleanliness. You know DET is equally good in hard or soft, warm or cold water. You can even clean floors, sinks, crockery, cutlery, etc. gleaming bright with DET.

Swastik Oil Mills Ltd., Bombay

Shilpi S.O.M.66

OVER with a pinch of snuff!

Before the discovery of modern anaesthetics, the surgeons of the eighteenth and nineteenth centuries sometimes intoxicated their patients with alcohol or, when the operation required freedom from struggling, even drugged them with narcotics.

With all this, the surgeons primarily depended on speed—an operation often was a sleight-of-hand affair to minimize the suffering of the patient. Langenbeck, Surgeon-General of the Hanoverian army in the time of Napoleon was credited with the ability to amputate a shoulder while one might take a pinch of snuff!

Today, I.C.I. serves the surgeon with safe and efficacious anaesthetics they can use with perfect confidence—'Fluothane' and 'Trilene' for anaesthesia by inhalation; 'Kemithal' for intravenous administration.

IMPERIAL CHEMICAL INDUSTRIES (INDIA) PRIVATE LIMITED

ICI

ICP 836

PORTRAIT OF A COMPANY*

1. The Upjohn Company**

Kalamazoo, Michigan, U.S.A.

The Upjohn Company, like most of the other leading pharmaceutical houses, dates from a small business founded on a pharmaceutical speciality. In this case it was a friable pill manufactured by a patented process, developed by the founder Dr. W. E. Upjohn. These pills were advertised and sold to the medical and pharmaceutical professions under the slogan, "Can be crushed to powder under the thumb." This statement combined with an illustration of a pill and thumb made up the Company's first trade mark.

Dr. Upjohn's early experiments on the friable pill were carried out in Hasting, Michigan, where he was a practising physician. In 1886, he moved to Kalamazoo where, a year later, with his three brothers the business was incorporated as the "Upjohn Pill and Granule Company." Much of the early pill manufacturing, coating, and bottling equipment was designed by the Upjohn brothers and modifications of this equipment are still in use.

Competition forced the Company to enlarge its scope and general pharmaceuticals were added to the line. In 1908, *Phenolax Wafers*, first of the candy-type laxative tablets containing phenolphthalein, was added, and this gave a stimulus to the growing business. In 1921, *Citrocarbonate*, best known of the Company's line of effervescent salts, and an improved form of digitalis, were developed. In 1929 a standardized high-potency cod liver oil was introduced. This was followed, in the next few years, by such well known nutritional products as *Myeladol*, *Accesserone*, and *Vitrate*, and *Jeculin* an iron and liver tonic. All these and numerous other preparations covering nearly the whole range of drug therapy are now listed in the Upjohn catalogue.

In 1909 the Upjohn Company occupied seven buildings in downtown Kalamazoo. By 1945, the Company occupied at its several sites in the city, thirty buildings aggregating a floor area of 19 acres. The more recent building activities were begun in 1934 with the erection of a concentrate plant for cod liver oil. The following year a new office building was completed, and the growing needs for power and heat were met with a new and modern power plant. In 1936 a new combined manufacturing plant and tower were built. Between 1937 and 1941, warehouses with railroad sidings were completed covering 200,000 square feet. In 1943 a soluble elastic capsule plant was built, and in 1944 a factory was purchased and re-equipped for the manufacture of penicillin. A new plant with floor space of 33 acres, designed for maximum efficiency and flexibility to meet needs of future expansions, was completed in 1951 in collaboration with engineers of the Austin Company. Several smaller buildings including the antibiotics and fine chemical buildings are adjacent to the main plant. With these modern manufacturing facilities, production capacity was increased by more than 50 per cent. There is a continuous line of production starting with raw materials brought directly to one side of the building on a railroad spur, moving across the plant through various stages of production and terminating as finished, packaged pharmaceuticals ready to be sent to the branch offices. Large scale production of newly discovered and developed drugs may be started within a matter of hours. The Company recently expanded its Veterinary Research programme and has an experiment station north of Kalamazoo devoted to the

* In this series will be published brief descriptions of the development of antibiotics manufacturing firms of the world.

** Courtesy: The Upjohn Company, Kalamazoo, Mich.

study and evaluation of veterinary products. The Agricultural Research and Development area has 43 acres of planted land for routine screening. Many new compounds are tested for their activity as pesticides, fungicides, bacteriocides and for their growth regulatory properties.

Expansion in products and facilities has been accompanied by expansion in personnel. From a one-man force in 1886, the Company has increased its personnel until today it numbers around 5,250. Of these about 3,300 are located in Kalamazoo, the rest in various branch offices. The fact that there has never been any "labour trouble" can be attributed to the foresightedness of the founder and those who have followed him in top management. A comprehensive insurance programme including group life, hospitalization, surgical, and retirement annuity has been in force for several years.

Distribution of Upjohn products is through four channels: Wholesale druggists, retail pharmacists, hospitals and physicians. To handle this distribution, the Company has 18 branch offices in the United States and subsidiary companies in Canada (1935), England (1953), Brazil (1954), Mexico (1955), Australia (1956), France, South Africa, Japan, Columbia, Panama and Puerto Rico. With six of these, England, Canada, Australia, France, Japan and Brazil, pharmaceuticals are manufactured under rigid specifications and controls maintained by the parent company. With a further renewed impetus from foreign demands on the Company during World War II, an active Upjohn International Division is in operation and foreign business is being fostered with considerable success.

One of the notable characteristics of the pharmaceutical industry is the number of new products introduced each year and the comparative brevity of their existence on the market due to displacement by better ones. This does not imply that all older products are obsolete, for that obviously is not the case. The vitamins, sulfas and penicillin, to mention only a few, are still going strong. And three products offered in the first Upjohn catalogue in 1886 are still listed in the current catalogue. It does mean, however, that there is a tremendous effort exerted in the industry to discover new therapeutic agents and to improve formulations of existing ones, an effort which creates rugged competition but works to the advantage of those who need and will use the new products. It also means that without research a company would be relegated to a minor position, even if it were fortunate enough to survive. The pharmaceutical industry, therefore, places a premium on research and it behoves a company to have an excellent research organization, for new and better products are the result of research.

Because of public campaigns to raise money for research on certain diseases there may be an impression that research and money are synonymous, that a solution to any problem is automatically assured provided enough money is forthcoming. Money is essential, of course, but it can be effective only when translated into buildings, equipment and personnel, the ingredients necessary for research, and even then, only if someone has a good idea.

The Upjohn research organization is liberally supplied with all of these essentials. Research and development activities are housed in 22 buildings and occupy over 7.5 acres of floor space. Replacement cost of this housing is estimated at upward of 14 million dollars.

These buildings are furnished with all sorts of equipment and facilities for the conduct of a wide variety of research projects. Among the many hundreds of the usual, more or less prosaic, items are many types of apparatus which permit scientists to probe more deeply into the behaviour

of matter, both animate and inanimate, than they could without instrumental assistance. Some of the more interesting of these are the Craig counter-current apparatus, the electron microscope, gas chromatography apparatus, infrared and ultraviolet spectrophotometers, the Van de Graaff electron beam accelerator, the ultra-centrifuge, the moving boundary electrophoresis apparatus, the polarograph, X-ray diffraction apparatus, and light scattering apparatus. Also of interest are the automatic recording titrator, the nuclear magnetic resonance spectrometer, the rotary dispersion spectrometer and the spectral photofluometer. In addition, although automation in research may never be possible, instrumentation research at the Upjohn Company has produced an automatic chromatography fraction cutter (dubbed the mechanical chemist), an automatic recording nephelometer (the mechanical bacteriologist), and an analog computer for special process engineering problems. No possibility of instrumental assistance is overlooked if it will enable scientists to carry out their investigations more effectively.

An adjunct which is essential to the conduct of research is a library which provides access to the vast amount of scientific knowledge. The Upjohn library has a collection of approximately 26,000 bound books and periodicals of which books comprise about a third. In addition there are many unbound periodicals, patents, pamphlets, photostats and microfilms. The library subscribes to 1800 journals covering many branches of science, and the number is constantly increasing as research interests require additional journals and as new journals are founded. Moreover, there are many supplementary services which a library can provide in addition to documentary material, and scientists need all of these in their efforts to keep abreast of the latest discoveries and developments in their field.

While buildings and equipment provide facilities for the conduct of research, they do not and cannot provide the ideas upon which all research is based. For that purpose people are needed. From a numerical standpoint the Upjohn research organisation was anything but impressive when it was established in 1913, for it consisted of only one man. (And it may be interesting to note that this same man, although retired, now serves as Research Consultant to the President). At present there are 300 technical people engaged in research and development, and an almost equal number of non-technical people in supporting roles. In the group of 300 technical people is a diversity of talents and abilities—engineers, medical men, veterinarians and scientists representing many branches of the physical and biological sciences. Specialists in these sciences and in ever-narrowing scientific knowledge which has accumulated is making it futile for any one man to keep up with, let alone assimilate, the advances in a broad scientific area.

In order to cope with the complexities of pharmaceutical research, groups of specialists in different sciences are formed for the purpose of solving problems, this is the so-called team approach, and it is the most effective way of dealing with these problems. Although it is still true that an idea is born in the mind of an individual, the efforts and co-operation of many different scientists are often required to develop the idea and bring it to fruition. Research and development activities at the Upjohn Company are conveniently separated into divisions, departments and sections primarily for administrative purposes, but in tackling problems, demarkation lines are ignored for the emphasis is then on integration and co-ordination of efforts for the attainment of a common goal.

As would be expected from the diversity of their backgrounds, Upjohn scientists are interested in many different research areas. One area of major interest is antibiotics, and an intensive search is made to find new ones. Such a search may mean the screening of hundreds of thousands of soil samples and the expenditure of years of effort and millions of dollars, all with absolutely no assurance of success. Antibiotics research involves extremely unfavourable odds, and not every company is equipped to make the gamble. Upjohn now markets a long list of penicillin, tetracycline, bacitracin, albamycin, neomycin, and sulfa preparations. Among these are the long-acting preparation *Depo-Penicillin*, mixtures of penicillin and sulfonamides such as *Sulfa-Sugracillin* and *Biosulfa*, bacitracin preparations, combinations of antibiotics and hormones such as *Neo-Cortef* and *Neo-Delta-Cortef*. *Cer-O-Cillin* (Penicillin O), which has low allergenicity, is another important Upjohn product.

Hormones have long been a subject of interest in Upjohn research. Upjohn is, the world's chief producer of adrenal cortical extracts. The Company pioneered research on the application of micro-organisms to steroid hormones and demonstrated that micro-organisms can function as reagents to perform highly specific chemical operations. When cortisone was first discovered it cost $ 68,000 per pound. Upjohn's research team was able to synthesize cortisone from the Mexican yam which brought the price down to $ 3,000 per pound. In the field of sex hormones the Company has developed long-acting suspensions of the male and female hormones, which cut materially the number of injections needed for effective treatment. The most recent discovery is *Halotestin*, an oral androgen, which is five times more effective than other male hormones available.

Vitamins, too, are an area of traditional interest, and formulations of vitamin products are constantly revised in keeping with the most recent knowledge concerning them. Also under investigation are those medical problems in which an important role may be played by an alteration or defect in nutrition or metabolism. Upjohn Company now markets more than 50 different vitamin preparations.

Among other research interests are such subjects as cancer, mental disease, hypertension, diabetes, atherosclerosis, edema and congestive heart failure, gastric ulcers, relief of pain, and virus and fungus infections. Recent achievements in these fields include the oral antidiabetic drug *Orinase* which was developed and clinically tested in the United States by the Upjohn Company. The Company's chemists have also succeeded in employing beef bone gelatine to produce *Gelfoam* and *Gelfilm*, sponges that are used to halt bleeding.

Then, too there are such varied interests as agricultural chemicals, veterinary medicine, mechanism of drug action, assays, analytical methods, fermentation processes, exploratory chemical engineering, improved modes of drug administration, and ways and means of improving pharmaceutical elegance, a long characteristic of Upjohn formulations.

It takes money to operate an extensive research programme, and the portion of the Upjohn budget spent on research is comparable to the amount spent on food in some family budgets. That such a comparison can be made is quite appropriate, for what food is to a family, research is to a pharmaceutical company, each is an item essential for existence. Productivity of Upjohn research is attested to by over 700 products it has placed on the market, by more than 400 patents which have been issued, and nearly 700 research articles covering many scientific areas. Although the pace of pharmaceutical research precludes complacency, the Upjohn Company regards the future with confidence.

2. The Royal Netherlands Fermentation Industries Limited, Delft, Holland

K. SCHEURKOGEL,
Department of Antibiotics, Koninklijke Nederlandsche Gist-en Spiritusfabriek N. V., Delft, Holland.

As far back as 1870 the "Koninklijke Nederlandsche Gist-en Spiritusfabriek N.V." (Royal Netherlands Fermentation Industries Ltd.) with its headquarters and mother-factory in Delft, was already engaged in the production of yeast and alcohol. Today the industrial activities of this Corporation, with factories in Belgium, Germany, Portugal and England, have expanded considerably.

Although World War II and the resulting occupation of Netherlands hampered the international growth of the company, it set upon itself a new and very ambitious task : the production of penicillin. Notwithstanding the severe handicap of being totally isolated from the scientific achievements in this field in England and the United States of America, the combined efforts of Dutch scientists and engineers paid off with a completely original process for the production of high purity penicillin salt on an industrial scale. This entirely independent development is all the more amazing when one considers the fact that the occupation forces in the Netherlands were not to profit by this discovery which for the same reason was a closely guarded strategic secret in the United States at that time. The secrecy with which this research was conducted in Delft was indeed successful.

Immediately after the war in 1945, the Royal Netherlands Fermentation Industries started building a new plant for penicillin fermentation, and the first batch of the antibiotic produced in the new factory was delivered to the Dutch hospitals by 1946.

Besides penicillin, the Corporation produces on a large scale streptomycin and synthetic chloramphenicol. The capacity of present day Dutch antibiotics industry is far in excess of the home demand so that a large part of the production is exported directly to other countries as well as to Dutch companies' own packaging plants overseas.

The Royal Netherlands Fermentation Industries have also a heavy research programme particularly for the discovery of new antibiotics. The research facilities of the Corporation were recently expanded with the addition of a Research Fermentation Pilot Plant unit. These factory-sized pilot plants facilitate investigations under circumstances and conditions prevailing in full-scale industrial production. Without interfering with the line of regular production in the parent factory, these research plants can produce simultaneously sufficient quantities of various compounds for clinical tests. In one of their Research Plants some 27 all stainless steel fermentors of capacities 5, 25, 150 and 800 gallons are continuously in operation. The general layout of these fermentors is such as to make the handling of the numerous valves as easy as possible and resulted in placing most of the valves neatly arranged on one side of the fermentors, the space between two rows of fermentors being known as the "valve lane". The

pilot plants have been designed not only to study fermentation problems on the intermediate level between laboratory and production, but are also fully equipped for producing any research quantity of a new fermentation product for industrial or clinical evaluation. In addition to studies on production problems relating to penicillin, streptomycin, riboflavin and steroids (products already in regular production), intensive studies are underway on the production of Pimaricin, a new antifungal antibiotic, developed in the Corporation's own laboratories.

As the Royal Netherlands Fermentation Industries earned its name with an achievement under the most difficult and unfavourable circumstances, one may have confidence in the capability of their highly developed Research Division to succeed in the accomplishment of further technical and scientific discoveries which may add still more lustre to their nearly ninety years old motto : Science, Quality and Service.

The Priceless Ingredient

In the city of Bagdad lived Hakeem, the Wise One, and many people went to him for counsel, which he gave freely to all, asking nothing in return.

There came to him a young man, who had spent much but got little, and said: "Tell me, Wise One, what shall I do to receive the most for that which I spend?"

Hakeem answered: "A thing that is bought or sold has no value unless it contains that which cannot be bought or sold. Look for the Priceless Ingredient."

"But, what is this Priceless Ingredient?" asked the young man.

Spoke then the Wise One: "My son, the Priceless Ingredient of every product in the market-place is the Honor and Integrity of him who makes it. Consider his name before you buy."

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